GRAS MONOGRAPH SERIES

CARAMEL

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SUMMARY

Description and Specifications

Caramel coloring is a complex polysaccharide of indeterminate formula prepared by heating a food-grade carbohydrate such as dextrose, invert sugar, or starch in the presence of a catalyst. The latter may include acetic, sulfurous, or citric acid, alkalies such as ammonium, calcium, or sodium hydroxide or their salts (038). According to the Federal Code of Regulations (038), caramel coloring may not contain more than 10 ppm lead, 3 ppm arsenic, and 0.1 ppm mercury. Ash content should not exceed 8% according to the National Formulary (112). Additional specifications will vary according to the manufacturer's requirements, the pH falling between 2.7 and 4.5 and specific gravity averaging 38° Bé.

Attempts to analyze caramel have rarely produced results that can be duplicated because the composition of the coloring will vary with the temperature and the method of preparation (132). About 50% of the coloring is digestible carbohydrate, 25% is nondigestible carbohydrate, and 25% is melanodins (010).

Acute Toxicity

Acute toxicity levels for caramel have not been established. Rats fed 30 ml caramel per kilogram body weight in a single dose did not exhibit systemic damage or microscopically observable pathology that could be attributed to the coloring (032). On the basis of this study the LD_{50} can be expected to exceed 30 ml/kg body weight in rats.

Short-terms and Long-term Studies

Toxicological studies concentrated on oral administration of caramel to small mammals. Caramel coloring was obtained from starch hydrolysates with ammonium hydroxide, sodium hydroxide, bisulfite salts, and sulfurous acid catalysts. Short-term and long-term studies (031, 051, 067) all indicated that as much as 10 g/kg body weight could be ingested daily by rats without causing observable damage or significant changes in blood or urine. The only study conducted on an individual human indicated that 120 g per day of pure caramel had to be consumed before metabolic changes were reflected in the urine (012).

want to sell their products at a very low price. It goes without saying that cheaper grades of caramel are not processed or refined as carefully as the better quality grades (094). Caramel coloring derived from a mixture of residual sugar greens or from low-quality products of the refining process should be evaluated for safety more carefully than that derived from high-quality dextrose.

CHEMICAL INFORMATION

I. Nomenclature

A. Common names: caramel color, burnt sugar color, brewers' caramel, beverage caramel, bakers' or confectioners' caramel, double-strength distillers' caramel.

B. Chemical name: none.

C. Trade names: caramel E, caramel AB, etc. These consist primarily of company codes differentiating batches of coloring.

D. Chemical Abstracts Number: MX 8028895

II. Empirical Formula

It is difficult to reduce the chemistry of caramel to simple terms for, during polymerization, simple sugars are formed into small and large sugar complexes. It should be noted that the term caramel as used commercially refers to caramel coloring, not to the caramel flavoring produced when a housewife heats sugar to the browning point. The impossibility of duplicating commercial methods in the laboratory (and the secrecy which producers maintain), not to mention the complexity of the substance itself, makes it difficult to obtain results that are reproducible.

A full review of early research on the structure and chemical composition of caramel is available (157). Eugene Peligot in 1838 gave the name caramel to a substance he found mixed with sugar; Gelis in 1848 confirmed Peligot's work and extended it, breaking caramel down into three substances--caramelan, caramelen, and caramelin (157). Subsequent research on the chemistry of caramel was carried out by Stolle, Trillat, Ehrlich, Green and others. In 1917 Cunningham and Doree (157) proposed formulas for the components isolated by Gelis and later work with purer compounds established the complexity of products resulting from the polymerization of sugar.

Polymerization through thermal action alone yields reductones, an oxycarboxyl group, and the furanic derivatives furfurole and alpha-oxymethylfurfurole. Polymerization in the presence of catalysts, as in commercial production, yields reductones, aldehydes, cyclopental, cyclohexyl structures such as quinone, furfurole, oxymethylfurfurole; also

amino pyrrole, imidazole, pyridine, pyrazine, and imino derivatives (157). In addition to these compounds, caramel made from refined sugar will have as coloring matter, nitrogen-free compounds, melanoidins (138), iron complex coloring compounds, and the fractions designated isosaccharosan, caramelan, caramelen, and caramelin (005).

III. Structural Formula

The complex and indeterminate nature of caramel precludes a specific structural formula. However, acid-base titration curves indicate the presence of four definite reactions for which tentative structural interpretations have been made. The pK's representing sulfonic acid ionization, quaternary ammonium ionization, secondary amine dissociation, and -OH dissociation-ionization, respectively are 3.6, 6.1, 9.0, and 11.2. Seventy percent of the sulfur is in the form of a titratable group while only 12% of the nitrogen present is in the form of a secondary amine. Apparently one -OH group is present per six carbons and a formula weight of 130 is found with $C_6H_5O_2$ $(S_{.043}O_{.073})$ $(N_{.16}H_{.16})$ where the sulfonate replaces an -OH and the secondary amine replaces an ether bridge (004).

IV. Molecular Weight

The molecular weight of a specific caramel color can be obtained by measuring the diffusion coefficients of fractions precipitated with ethyl alcohol. Using the Einstein equation and these diffusion coefficients along with the measured partial specific volume, molar volumes can be calculated. Following these methods, the average molecular weight for 28% caramel is 78,000 with 40% hydration; for 18% it is ca. 10,000, 23%, 5000, and the remainder, ca. 500. High-speed centrifugation has given a molecular weight of over 1 million for 0.7% caramel. With a 75% dialysate, it is estimated that 24% of the caramel has a molecular weight over 12,000 (004).

V. Specifications

A. Chemical: Chemical specifications for caramel will differ according to its intended use (see Section VI.B). Some sample commercial specifications* are:

Caramel Powder:	Moisture	3.0% maximum
	Total ash	10% maximum
	Water insolubles	0.5% maximum
	Color	62 ± 3% transmission
	Extraneous matter	
	Macro	None
	Micro	None
	Microbiological examination	
	Standard plate count	1000/g maximum
	Yeast-mold count	100/g maximum
	E. coli	Negative
Caramel Liquid:	Total solids	66-67% (36° Bé at 60°F)
	Total ash	3.5% maximum
	pH (as is)	2.90 ± .05
	Viscosity at 80°F	160 ± 10 cps
	Acid boil test	A rating after 24 hr
	Iron	10 ppm maximum
	Copper	1.5 ppm maximum
	Lead	2 ppm maximum
	Color (0.1% soln.)	
	Wavelength	% transmission
	600 mμ	75
	500 mμ	43
	400 mμ	8
	Extraneous matter	
	Macro	None
	Micro	None

* Specifications are taken from confidential manufacturers' reports furnished as part of an NAS/NRC Food Protection Committee survey.

	Microbiological examination	
	Standard plate	1000/g maximum
	Yeast-mold count	100/g maximum
	E. coli	Negative
Double-strength Distillers Caramel:	pH as is	2.5 minimum - 4.5 maximum
	Ash	0.2% maximum
	Iron	5 ppm maximum
	Copper	5 ppm maximum
	Color	300 Klett units
	Viscosity	Fluid
	Solubility	Immediate (1 g in 100 ml of 110-proof spirits)
	Stability	No turbidity or flocculation after 24 hours at room temperature.

B. Food Grade: The Code of Federal Regulations (038) specifies that caramel may be made from food grade dextrose, invert sugar, lactose, malt syrup, molasses, starch hydrolysates and fractions thereof, and sucrose. Caramelization of these substrates may be assisted by the following substances in amounts consistent with good manufacturing practice: acetic acid, citric acid, phosphoric acid, sulfuric acid, sulfurous acid, ammonium hydroxide, calcium hydroxide (U.S.P.), potassium hydroxide, sodium hydroxide, and the salts comprising ammonium, sodium or potassium carbonate, bicarbonate, phosphate (including dibasic phosphate and monobasic phosphate), sulfate and sulfite.

This regulation goes on to state that certain polyglycerol esters of fatty acids identified in the regulations may be used as antifoaming agents in amounts not greater than that required to produce the intended effect. The Code also states that color additive mixtures for food use made with caramel may contain only diluents that are suitable and that are listed in this subpart as safe in color additive mixtures for coloring foods.

The Code sets forth specifications for limits on lead, arsenic and mercury:

Lead (as Pb) ---------- Not more than 10 parts per million
Arsenic (as As) ---------- Not more than 3 parts per million
Mercury (as Hg) ---------- Not more than 0.1 part per million

Finally, the Code states that caramel may be safely used for coloring foods generally, in amounts consistent with good manufacturing practice, except that it may not be used to color food for which standards of identity have been promulgated under a stipulated section of the act unless added color is authorized by such standards.

British Standard 3874 (022) specifies that the density of a 10% w/v solution of caramel shall be not less than 1.0213 g/ml at 20°C. Color intensity of the caramel shall be not less than 20,000 EBC* units. Caramel intended for use in foods should not contain more than 20 ppm copper calculated on a dry basis, 3 ppm arsenic, or more than 5 ppm lead.

The National Formulary (112) specifies that 1 part of caramel dissolved in 1000 parts water should yield a clear solution with a distinctive yellowish-orange color which remains unchanged, with no precipitate after exposure to sunlight for 6 hours. Caramel spread in a thin layer on a glass plate appears homogeneous, reddish-brown, and transparent. Caramel is miscible with water and is soluble in dilute alcohol up to 55%. It is not soluble in ether, chloroform, acetone, benzene, and solvent hexane. Specific gravity should be not less than 1.30, and the addition of 0.5 ml of phosphoric acid to 20 ml of a solution of caramel should produce no precipitation. When incinerated, caramel swells and forms a cokelike charcoal that burns off after prolonged heating at a high temperature. It should yield not more than 8% ash.

VI. Description

A. General Characteristics

Caramel is an amorphous, dark-brown substance with a characteristic odor of burnt sugar and a taste that varies from sweet to bitter, depending on its pH. It is most commonly manufactured as a viscous liquid, but solid and powdered caramels are also available. The solid form is reddish-brown, brittle, amorphous, and highly deliquescent (018).

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* European Brewery Convention

Caramel is a colloidal aggregate that is dispersible in water, partially dialyzable, and partly dispersible in alcohol-water solution. Its isoelectric point and pH can vary widely. Approximately 50% of the coloring is digestible carbohydrate, 25% is nondigestible carbohydrate, and 25% is melanodins similar to those found in roasted coffee or broiled meat (010).

There are three basic groups of caramel: cane sugar, which has a very low tinctorial power, but which will withstand concentrations of alcohol as high as 190 proof; malt caramel, which is prepared from malt syrup or a combination of malt syrup and corn sugar syrup; and corn sugar or dextrose caramel. This last is the important commercial coloring used in the United States and other countries (049).

Caramel coloring may further be classified by the type of catalyst used to aid production of color aggregates. These are alkali caramels (ammonia or a caustic soda) and ammonia-sulfur dioxide caramels (018). The major reactant may consist of any food-grade reducing sugar. Additives, which must also be food grade, include acetic, citric, phosphoric, sulfuric, and sulfurous acids, and ammonium, potassium, or sodium hydroxides, various salts, and a limited amount of polyglycerol esters of fatty acids (018).

B. Physical Properties

At 500 mμ the coloring power for all polymerized substances in caramel is equivalent on a per gram basis. Intrinsic viscosities have been measured on fractionated and dialyzed caramels and indicate that caramel is primarily spherical in nature and has the property of a dispersed polyelectrolyte, the solubility of which is dependent upon the hydration of ionized polar groups (004).

The physical properties of any caramel vary according to its commercial use. Among characteristics taken into account when a manufacturer chooses a caramel coloring are tinctorial power, specific gravity, pH, ash content, foaming properties, acid fastness, alcohol stability and isoelectric point (049).

Tinctorial power is one of the most important properties of caramel coloring. Usually this is determined by diluting 1 g of the coloring in 1 liter of water and measuring the solution against a Lovibond No. 52 caramel glass slide. Spectrophotometers and colorimeters are also used when available. The best wavelength for measurement is from 590 to 640 mμ. A recent attempt has been made to establish coloring power by measuring the extinction of monochromatic light. (Wavelength of 0.4 - 0.7μ) over a concentration range of 0.02 - 2.5 g caramel per 100 ml water (063). A satisfactory tinctorial power varies according to the user, bakers and confectioners requiring a higher tinctorial power than brewers and soft drink manufacturers.

Specific gravity may vary from 30° to 40° Bé, but average 38° Bé. The variation is caused by attempts to adjust solids, tinctorial power, and viscosity. The last is important, for if a caramel is too thick it will not flow, but if it is diluted too much it loses tinctorial power. A good quality acid-proof caramel color should weigh about 11 lb. per gallon and have a Bé of 34.5 - 36.5° at 60°F.

The pH varies from 2.7 - 3.3 for acid-fast beverage color to 3.2 - 3.6, for foaming beverage caramel and 4.0 - 4.5 for bakers' and brewers' colors (049). Above a pH of 6.0 the coloring is susceptible to mold while if the pH falls below 2.5 the caramel tends to resinify.

The ash content depends on the content of the starting sugar. No. 70 dextrose contains about 0.6% ash on a dry basis, but this may be increased by condensation or salts used as catalysts (049).

Manufacturers of soft drinks must consider the foaming properties and the acid fastness of a caramel coloring. The latter property ensures that the coloring will not floc or precipitate in an acid beverage.

Colors used in imitation vanilla extract and pharmaceutical syrups and extracts must have alcohol stability. This is tested by adding 1 g of caramel color to 100 ml of an alcohol solution. A good caramel will remain clear indefinitely in 70% alcohol.

Caramel, as a colloid, carries an electrical charge, the nature of which depends on the manufacturing method. If the pH of the caramel is above its isoelectric point, the caramel is negatively charged; below, it is positive. In order to prevent precipitation in soft drinks such as root beers and colas, the caramel must have a strong negative charge and its isoelectric point should be at pH 2 or less (056).

C. Stability in Containers

Caramelization continues after processing, although at a slow rate, and the coloring should be stored at a cool temperature. A quality caramel may keep for over a year without thickening if stored properly (128). Liquid caramel must be stored in stainless steel tanks or polyethylene-lined drums. Powdered caramel is highly hygroscopic and must be packaged in polyethylene-lined drums having moisture barriers and vapor-tight covers and be stored in a cool, dry place.

VII. Analytical Methods

The analytical methods used for caramel fall into two classes - those used to detect caramel in food and those used to determine the constituents of the caramel itself.

There is no simple or unequivocal method for detecting caramel in foodstuffs, as the variety of European methods attests (079). In the United States, where the use of caramel in wines and whiskies is strictly limited, several methods have been developed. Most of those used by the Association of Official Agricultural Chemists are based on gel filtration with a variety of reagents, and partition chromatography or colorimetric analysis. The Mathers test, which can be used on beer, vinegar, cordials and flavoring extracts, uses successive precipitations and centrifugations with HCl-alcohol to obtain a residue indicating the presence or absence of caramel. Briefly, this test consists of the following procedure (169).

One milliliter of pectin solution (made by dissolving 1 gm of pectin in 75 ml of water and adding 25 ml of alcohol) is added to 10 ml of the sample to be tested. To this material, which is in a centrifuge bottle, 3 drops of concentrated HCl are added and the bottle is then filled with alcohol. It is shaken well and centrifuged for 5-10 minutes or more. The supernatant is decanted off the residue and the residue dissolved by adding 10 ml water and shaking. Three drops of concentrated HCl and 50 ml or more of alcohol are added; the bottle is shaken again and centrifuged. This process is repeated until the upper alcoholic layer is clear and colorless. After the final decantation the residue is dissolved in 10 ml hot water. A colorless solution indicates that no caramel is present. A clear brown liquid indicates caramel in most cases. This can further be confirmed by adding 1 ml of reagent (1 g of 2, 4-dinitrophenylhydrazine in 7.5 ml concentrated sulfuric acid brought up to 75 ml with 95% ethyl alcohol) to the residue in 10 ml hot water. The bottle is immersed in a beaker of boiling water for 30 minutes. When substantial amounts of caramel are present a precipitate is formed. For further confirmation when the amount of caramel is slight, the hot test solution can be poured on a small filter paper and washed with hot water. If caramel is present, a reddish brown precipitate will be seen on the filter.

The Mallory-Love test (099) uses organic acids and solvents to free caramel from other coloring matter. Stinson and Willits (150) developed a gel filtration method using cross-linked dextran that allowed them to separate caramel colors from sugar and salts in one pass through a large Sephadex column.

As indicated earlier, attempts to analyze caramel have met with limited success. The composition of a caramel varies with pH, temperature of heating, and the type of sugar used in preparing it. Thus, early attempts to break down caramel rarely produced results that could be replicated. Shumacher and Buchanan (145) in 1932 heated dry sucrose without a catalyst to make a systematic comparison of caramel. They prepared three series of caramels at 180°, 200°, and 210°C. By plotting loss of weight against time of heating, they established the points at which isosaccharosan, caramelan, and caramelen would form. Caramels of low molecular weight were found to have low color values and to dialyze largely - thus higher color values are produced by more complete hydration.

Thin-layer and paper chromatography of caramelized sucrose carried out by Kitaoka and Suzuki (086) gave a great number of close spots in a belt such that the spots could barely be distinguished from each other. They were able to isolate 15 compounds, including unchanged sucrose, hydrolysis products of glucose and fructose, dehydration products of levoglucosan, and three alpha-glucodisaccharides of isomaltose, kojibiose, and nigerose.

Ramaiah and co-workers (132) prepared caramel from glucose, fructose, sucrose, maltose, and liquid glucose. These caramels were separated by precipitation with alcohol and examined chromatographically with Whatman No. 1 paper and n-butanol-ethanol-water solvent. Four reducing compounds are shown with Rf values of (x100) 6.5, 4.35, 1.4, and 0. These appeared whether a single sugar or a mixture of sugars was caramelized. A spot at an Rf of 0 indicated the presence of one or more reducing substances of relatively high molecular weight. These authors compared caramel prepared by heating sugar at 200°C and by heating a sugar solution at 100°C in the presence of an alkali. Analysis by the above methods showed that sugar prepared without a catalyst had an additional reducing compound with an Rf of 8.7. The authors concluded that the composition of caramel varies not with the sugars, but with the method used to prepare it.

VIII. Occurrence and Levels

Since caramel is a manufactured substance, produced by heating sugars, it does not occur naturally in plants or animals. Potatoes will caramelize during frying (076) and evaporated milk stored for long periods develops a brown color and caramel flavor owing to the caramelization of lactose that is initiated during sterilization (046). No synthetic or natural inorganic sources were encountered in the literature.

BIOLOGICAL DATA

I. Acute Toxicity

One acute toxicity study was found in the literature. The caramel coloring tested consisted of two types of caramel, single-strength beverage and single-strength bakers' caramel, the first made from starch hydrolysates, the last from molasses. The catalysts used were ammonium hydroxide, sulfuric and sulfurous acids, and sodium hydroxide. Thirty Sprague-Dawley rats were fed 20-30 ml/kg body weight in a single dose. The animals were observed for 14 days and then sacrificed. Microscopic examination revealed no systemic damage and the author concluded that the LD_{50} must exceed 30 ml/kg body weight (032).

II. Short-term Studies

The following summaries are representative of 11 studies examined in order to determine the effects of caramel coloring on small mammals.

A. Rats

1. Method (051)

Substance: Seven caramels obtained from starch hydrolysate; 102B, single-strength beverage, ammonium hydroxide, bisulfite salts, and sodium hydroxide catalysts; 103B, single-strength beverage, ammonium hydroxide and sulfurous acid catalysts; 104B, single-strength beverage, sulfuric acid, ammonium hydroxide, bisulfite salts, and ammonium sulfate catalysts; 107B, sulfuric acid, ammonium hydroxide, bisulfite salts and ammonium sulfate catalysts; 108B, residual sugar, no catalysts; 109B, acid-treated sugar, sulfuric acid and sodium hydroxide catalysts; 110B, double-strength beverage, ammonium hydroxide, bisulfite salts, and ammonium sulfate catalysts.

Species: Rats

Strain: Sprague-Dawley

Sex: M and F

Age at start of experiment: Not given

Duration of study: 21 days dosing plus 2 weeks observation = 35 days

Vehicle: Distilled water

Dose schedule: 0.5 ml caramel/day

Route: p.o. -- intubation

No. of animals per level: 10

Observations

Symptomatology: Some diarrhea, soft stools

Weight gain or loss: Comparable with controls

Survival: 99% (One death due to intubation difficulties)

Urine analysis: Not done

Hematology: Not done

Organ weights: Not given

Gross pathology: Kidneys, liver, spleen, heart, stomach, colon, small intestine examined for 16 animals. No gross pathology seen.

Microscopic pathology: Heart, kidneys, adrenals, and liver examined. Eight out of 16 animals (including controls) showed slight to moderate congestion of liver or spleen.

No reproductive indices, carcinogenic, teratological, or mutagenic observations made.

No significant adverse effects noted.

2. Method (031)

Substance: Caramel 25A-1, single-strength beverage obtained from starch hydrolysate with ammonium hydroxide, sulfurous acid, and sodium hydroxide catalysts; 30B-0 single-strength bakers' caramel obtained from starch hydrolysate with sulfuric acid and ammonium hydroxide catalysts; 30F-1, single-strength bakers' caramel from molasses with ammonium hydroxide and sulfurous acid catalysts.

Species: Rats

Strain: Sprague-Dawley

Sex: M and F

Age at start of experiment: Weanling

Duration of study: 90 days

Vehicle: Purina lab chow

Dose schedule: ad lib daily, 10 g/kg body weight of each of three caramel colors.

Route: p.o.

Number of animals per level: 20

Observations:

Symptomatology: None

Weight gain or loss: Comparable with controls

Survival: 100%

Urine analysis: 40 animals tested at beginning of experiment, and at 1, 2, and 3 months. Sugar, albumin, and acetone were either negative or trace.

Hematology: 40 animals tested at beginning of experiment, and at 1, 2, and 3 months for hemoglobin, hematocrit, WBC, and differential cell count. No significant differences noted.

Special tests: None

Organ weights: Not given

Gross pathology: 80 animals sacrificed and examined for gross systemic damage. All dosages 10 g/kg body weight except control.

Microscopic pathology: Small intestine, lungs, heart, adrenals, kidneys, pancreas, liver, spleen, stomach, cecum and colon sectioned and examined for 40 animals. Occasional lymphocytic peribronchiolar infiltration was noticed, along with some congestion in lungs, kidneys, or adrenal medulla in 8 out of 30 animals. However, 3 out of 10 controls showed the same congestion and 4 had peribronchiolar infiltration.

No reproductive indices, carcinogenic, teratological, or mutagenic observations made.

Special observations: The authors found that the cumulative efficiency of food utilization was lower for the experimental groups than for the control and that the percent of efficiency varied from 76.35 to 81.08 compared to controls. They infer from this that caramel colorings have a questionable nutritive value, if any. While weight gains in the experimental group were less than those of the controls, food consumption was greater.

3. Method (030)

Substance: Two double-strength beverage caramels derived from a starch hydrolysate were used. Catalysts were ammonium hydroxide, sulfurous acid, and bisulfite salts.

Species: Rats

Strain: Sprague-Dawley

Sex: M and F

Age at start of experiment: Weanling

Duration of study: 90 days

Vehicle: Purina lab chow

Dose schedule: 10 g/kg; 5 g/kg body weight

Route: p.o.

Number of animals per level: 20

Observations:

Symptomatology: None

Weight gain or loss: Comparable to controls

Survival: 100%

Urine Analysis: 60 animals tested at beginning of study and at monthly intervals thereafter for albumin, acetone, and sugar. No significant abnormalities noted.

Hematology: Blood tests made at beginning of study and at monthly intervals on 60 animals for RBC, hemoglobin, hematocrit and WBC and differential count. No abnormalities noted.

Organ weights: Not given

Gross pathology: 30 animals sacrificed and examined for gross systemic damage. None noted.

Microscopic pathology: Liver, spleen, pancreas, lungs, kidneys, brain, heart, gastrointestinal tract, lymphoid elements, adrenals, thyroid and parathyroids examined microscopically. No abnormalities as compared with controls noted.

No reproductive indices, carcinogenic, teratological, or mutagenic observations made.

B. Dogs

1. Method (072)

Substance: Single-strength acid-fast caramel

Species: Dog

Strain: Beagle

Sex: M and F

Age at start of experiment: Adult

Duration of study: 90 days

Vehicle: Purina dog chow, beef, and corn oil mixture

Dose schedule: 6.0, 12.5, and 25% of sample by weight in lab chow.

Route: p.o.

Number of animals per level: 6

Observations:

Symptomatology: None

Weight gain or loss: Comparable to controls

Survival: 100%

Urine analysis: 24 animals tested for reducing substance, albumin, and microscopic elements at beginning of study and 45 and 90 days following start of test. No abnormalities noted.

Hematology: Hemoglobin concentration, erythrocyte count, and total and differential leukocyte counts made at the beginning and at 45 and 90 days following start of test. No abnormalities noted.

Special tests: Liver and kidney function tests were conducted at the same time intervals as the urine and blood tests. There was no evidence of organ dysfunction.

Organ weights: Not given

Gross pathology: All body organs and glands examined for 24 dogs fed 6.0%, 12.5% and 25% caramel w/w. Findings for test dogs were comparable to those noted among controls.

No reproductive indices, carcinogenic, teratological, or mutagenic observations made.

III. Long-Term Studies

A. Rats

1. Method (067)

Substance: Three caramels obtained from starch hydrolysates with ammonium hydroxide and sulfurous acid catalysts. Three obtained from starch hydrolysates with ammonium hydroxide, sulfurous acid, and sodium bisulfite catalysts.

Species: Rats

Strain: Wistar

Age at start of experiment: Weanling

Duration of study: 300 days

Vehicle: Tap water

Dose schedule: 30 ml 10% caramel solution; 30 ml 20 % solution; or 2.7 g/day and 5.4 g/day

Route: p.o.

Number of animals per level: 10

Observations:

Symptomatology: None

Weight gain or loss: Comparable with controls

Survival: 100%

Urine analysis: Not done

Hematology: Hemoglobin, RBC, WBC, neutrophils, basophils, eosinophils determined for 10% and 20% levels with varying number of rats.

Special tests: None

Organ weights: Not given

Gross pathology: Not given

Microscopic pathology: Liver, spleen, kidney, stomach, heart, lungs, pancreas, gonads, duodenum, small intestine, and large intestine examined on 24 representative animals for both dosage groups. No damage seen that was considered to be caused by ingestion of caramel.

Number of generations and litters in a reproductive study: Records kept but not reported in study. No appreciable difference seen in size and number of litters between test animals and controls.

Reproductive physiology: None given

No carcinogenic, teratological, or mutagenic observations made.

Special observations: Absorption studies were carried out. These will be discussed in Section II of Biochemical Aspects.

Remarks: The authors selected 50 rats which were the offspring of the test animals in the experiment described and repeated their experiment using a 10% caramel solution for 100 days. At the end of this period, sections from 4 animals were fixed for histological examination and blood counts taken for 8 animals. Growth curves and blood counts for the second generation were normal. Histological examination of tissues revealed no abnormalities which the authors could construe as having been caused by the ingestion of caramel.

IV. Special Studies

The process used to combine ammonia and corn syrup solids or invert sugar to produce caramel coloring is similar to that used in producing ammoniated feed supplements from agricultural sugar (011). Consequently, concern was aroused when several cases of hysteria and convulsions were reported in farm animals, particularly beef cattle, that were fed ammoniated

molasses. Manufacture of ammoniated molasses subsequently has been abandoned because farmers persisted in feeding excessive amounts of the low-cost dietary supplement (011).

Wiggins in 1955 used chromatographic analysis to determine the substances produced during ammoniation of molasses. He isolated imidazole and pyrazine derivatives and identified 4(5)-methylimidazole (4-Me-I). He did not take his analysis further (011).

In 1969 Nishie and her co-workers (115) reopened investigation of the toxicity of compounds formed by interaction of ammonia with reducing sugars. Of the large variety of imidazoles and pyrazines tested, only 4-Me-I was found to have toxic effects.

Nishie et al. used male albino mice (20-25 g) to determine the median convulsive dose (CD_{50}) and the median lethal dose (LD_{50}) of the imidazoles, the median protective dose (PD_{50}) of anticonvulsants against a 90% convulsant dose of 4-Me-I, and the effects of 4-Me-I on spontaneous motor activity. Their findings on CD_{50} and LD_{50} are summarized in the table (see original paper for motor activity and anticonvulsant effects). All the imidazoles tested produced varying degrees of tremor, running, restlessness, sialorrhea, Straub tail, opisthotonus, and tonic extensor seizure that ended in death.

Convulsant and Lethal Effects of
Imidazoles, Pentylentetrazole, and Pyrazine in Mice

	pH (15mg/ml in H_2O)	$CD_{50} \pm$ SE(mg/kg)		$LD_{50} \pm$ SE(mg/kg)	
		i.p.	p.o.	i.p.	p.o.
4-Methylimidazole (4-Me-I)	8.2	155 ± 5	360 ± 18	165 ± 3	370 ± 15
1-Methylimidazole	9.2	380 ± 8.2	1400 ± 79	380 ± 8.2	1400 ± 79
2-Methylimidazole	10.2	500 ± 12	1300 ± 70	480 ± 18	1400 ± 114
Imidazole	9.8	560 ± 34	1880 ± 45	610 ± 7.4	1880 ± 45
Pentylenetetrazole	7.8	38.5 ± 3.4	75 ± 5.2	134 ± 7.4	214 ± 15.5
Pyrazine	6.8	Not convulsant		2000	2000

Nishie et al. also determined the CD_{50} and LD_{50} of 4-Me-I in 1-day-old chicks. The i.p. CD_{50} ($\pm$ SE) was 174 $\pm$ 10 g/kg; the LD_{50} was 210 $\pm$ 15 mg/kg. Per orally, the CD50 was 580 $\pm$ 30 mg/kg and the LD_{50} was 599 $\pm$ 50 mg/kg. Doses of 100 mg/kg i.p. caused tremors, peeping, and spreading of the wings. Doses greater than 150 mg/kg i.p. caused opisthotonus, prostration with clonic leg movements, and terminal tonic extensor seizure.

Rabbits were used to determine the effect of 4-Me-I on EEG, respiration, and ECG. Toxic manifestations included abrupt paralysis of respiration, cyanosis, and gradual cardiac arrest.

Seizures in mice were prevented by chlordiazepoxide and phenobarbital; these compounds given to rabbits at the onset of convulsions prevented epileptiform seizures and death.

Nishie et al. extended their work a year later to study the pharmacology of the alkyl and hydroxyalkylpyrazines produced in the ammoniation of glucose (116). They found that convulsions induced in mice by pentylenetetrazole and 4-Me-I were prevented by pyrazine and its hypnotic derivatives. This investigation provided further evidence that the chief toxic factor in ammoniated molasses is 4-Me-I. Nishie and co-workers concluded that since pharmacologically antagonistic methylpyrazines and methylimidazoles occur simultaneously in the ammoniation of glucose, the toxicity of the mixture depends on the relative ratios of these substances (116).

Remarks

The fact that none of these toxic manifestations have been seen in animals tested with caramel coloring may be owing to several factors: (1) Pure, simple carbohydrates are used in making caramel color while ammoniated feed is produced from complex mixtures of natural components in agricultural wastes; (2) These latter supplements are produced under alkaline conditions, caramel under neutral to acid conditions (Nishie's table shows that the higher pH values of the compounds tested produced CD_{50} values); (3) Animal food supplements such as ammoniated molasses

contain large amounts of imidazoles and pyrazines while caramel colors apparently do not contain these substances in significant amounts. Levels of consumption must also be considered in evaluating this information. A child that consumes five bottles of soft drink would be obtaining only 1/60th as much caramel color per kilogram of body weight on a nitrogen basis compared to an animal fed 1 lb. of ammoniated supplement at a level of 6-25% in a ration. (This is the ratio known to cause hysteria in some farm animals (011).

BIOCHEMICAL ASPECTS

I. Breakdown

Caramelization continues after the manufacturing process is finished, and after prolonged storage under extreme heat or dampness or other adverse conditions, caramel can resinify into an amorphous, irreversible gel that is not usable. It should be noted that caramels of a high acidity have a deteriorating effect on metals. This is counteracted by shipping the caramel in polyethylene-lined drums, but where the caramel is used in food products that are stored for a long time, particularly if the caramel is made from dextrose, it can be expected to accelerate corrosion of tin cans that do not have a protective lining (033).

II. Absorption-Distribution

There is very little available in the literature on the biochemical aspects of caramel. Haldi et al. (067) as part of a long-term experiment on toxicity carried out absorption studies with rats given 30 ml of 10% and 20% caramel solutions daily for 100 days. The feces were collected, leached, and caramel extracted. The difference in the amount of caramel in the feces and the amount ingested by each rat is reported as caramel absorbed. These workers found that while there were wide variations in caramel absorption from day to day, the average amount absorbed remained constant. They concluded from their limited observations that about one-third of the color-giving components of caramel may be absorbed by the albino rat (067).

III. Metabolism and Excretion

The metabolic studies that have been done on caramel were carried out with caramelized sugar heated without a catalyst. These studies were undertaken in order to find a suitable sugar substitute for the diabetic. Grafe and Schroeder (061) found in 1924 that the intestinal enzymes of the normal and diabetic man cannot split levoglucosan and 5-19% of levogluoside ingested appears in the urine. They found blood sugar slightly decreased and acidosis improved.

Grafe was one of the first workers to use caramel in treating diabetics. He found that caramel made by heating dry glucose to 146°C had a favorable effect on glycosuria. F.K. Bahrs, in preparing his doctoral thesis in 1940 (012) sought to determine the metabolic effects produced by substitutions of caramel for sugar. He prepared a diet for himself that had an albumin content of 56 g and a caloric value of 2300. The 410 calories of sugar in the diet were replaced by caramel. Bahrs followed this regimen for 20 days. He failed to find any increase in urine quotients except when large quantities of caramel (120 g) were consumed. The urine nitrogen was lower during the caramel periods. The author suggests that caramel excretion in the feces increased with larger doses (this has been observed in rats) and that this was the indirect cause of larger nitrogen losses.

More recently, Fournier (052) proposed that enzyme specificity may explain why caramel and dextrin, which are poorly defined structurally, are less easily metabolized than glucose and starch, their parent substances.

IV. Effects on Enzymes

No information

V. Drug Interaction

No information

VI. Consumer Exposure

A caramel manufacturers' committee estimated that 70,000 tons of commercial caramel colors are produced annually in the United States, a figure that suggests an average total consumption of approximately 0.85 g per person per day (010). However, a survey of 79 selected caramel manufacturers conducted by the NAS/NRC Food Protection Committee places the annual production for these firms at 11,500 tons (111). About 75% of the annual production in the United States is used in soft drinks. The amount of single-strength color used in beverages such as root beer and colas is less than 4 g/liter; for double-strength coloring it is 2 g/liter. This means that in a normal serving there is 1 g or less of color (010).

The Food Protection Committee of the National Academy of Sciences National Research Council suggests the following limitations on foods containing caramel: beverages, 2200 ppm; ice cream and ices, 590 ppm; candy, 180 ppm; baked goods, 220 ppm; syrups, 2800 ppm; meats, 2100 ppm (050).

In addition to soft drinks, caramel is widely used in other food products. These include fruit juices and juice drinks, baked beans, biscuits, bread, brown bread with as much as 5% of the total composition (094), butter, canned meat products, canned mushrooms and other vegetables, catsup, cheeses, condiments, confections, caramels, icings, gravies and sauces, fish pastes, ice cream and sherbet mixes, jams, jellies, marmalades, mincemeat, pet foods, pudding mixes, pickles and relishes, soups, table syrups and vinegars. While its use in the United States in alcoholic beverages is limited to blended whiskies and some beers, caramel is widely used in imported apertifs, brandies, cordials, rum, whiskey, and wines.

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Committee of Industrial Manufacturers and Users,
"A Review of Caramel Colors" (mimeographed report)

DESCRIPTION:

Caramel colors (caramels) are essentially colloidal aggregates that are dispersible in water, are partly dispersible in alcohol-water solutions, are only partially dialyzable, have some reducing capacity, have little or no taste at normal usage level, have isoelectric points and pH's varying over a wide range. Their colors are various shades of brown. In general, caramels used in alcoholic beverage, confections, baked goods and other solid foods have high isoelectric points, while those used in soft drinks have low isoelectric points.

Caramel colors may be classified into two major categories depending on the kind of catalyst that is used to aid formation of color aggregates. They are (1) alkali caramels (ammonia, caustic soda, etc.) and (2) ammonia-sulfur dioxide caramels. The pertinent difference between these types, so far as food use is concerned, is this: in solution at pH 2.5 the first group is positively charged while the second group is negatively charged. Any reducing sugar can be used as the major reactant. Food grade acids, alkalis and salts in various combinations may be employed to assist caramelization. Reactions may be carried out either at atmospheric or at elevated pressure. Reaction temperatures and times may vary depending on the nature of catalysts and pressures. Lists of permissible starting materials and catalysts are given in some national and international regulations. For examples, see references 1, 2 and 3.

The chemistry of caramelization is complex and is difficult to reduce to simple terms. It appears to be a type of condensation or polymerization in which the simple sugars are formed into larger units. Small amounts of simple combinations are also formed. In the case of reactions involving ammonia-sulfur dioxide catalyst, the same fundamental combinations occur with the ammonia promoting the color formation and the sulfur dioxide contributing a negative charge. Properly prepared caramel coloring contains only small amounts of ammonia or sulfur dioxide.

In typical products, approximately 50 percent of the material is still digestible carbohydrate, 25 percent is non-digestible carbohydrate and 25 percent melanoidins. These latter complexes are similar to those found in common foodstuffs, such as roasted coffee, broiled meats and baked cereal products.

Additional pertinent information on the types and on the physical and chemical characteristics of caramel colors is provided in references 4-11.

SPECIFICATIONS:

	1 Alkali Caramels	2 NH_3-SO_2 Caramels
Isoelectric point	>pH 2.5	<pH 2.5
pH	>3.0	>1.8
Solids content, %, min.	45	45
Ash, %, max. (a)	10 (b)	5 (b)
Nitrogen, %, max. (a)	4 (b)	3.5 (b)
Sulfur, %, max. (a)	2 (b)	5 (b)
Ammonia (N), %, max. (a)	0.5 (b)	0.5 (b)
Sulfur dioxide, %, max. (a)	0.1 (b,c)	0.1 (b,c)
Phosphorous (P_2O_5), %, max. (a)	0.5 (b)	0.5 (b)
Trace metals, mg. per kg.		
Lead		5
Arsenic		3
Mercury		0.1
Copper		20

In addition, the following, as sulfates, may not exceed 100 mg. per kg. singly or 200 mg. per kg. combined: antimony, chromium, zinc, barium. There should be no detectable traces of cadmium, selenium, tellurium, thallium, uranium, chromate or soluble barium salts.

(a) On a dry basis.

(b) These values are for caramels having color values of 20,000 EBC units. Higher values are permitted in proportion to color strength referred to the U.K. Standard method, 20,000 EBC (3, Appendix B) or other comparable methods.

(c) According to the method of Burroughs and Sparks. (22)

ADDITIONAL INFORMATION:

A. Uses of caramel colors.

Caramel colors are permitted for use universally in many different foods and beverages. Items that have been mentioned in several publications, including government regulations, are:

Food beverages.
- fruit juices and juice drinks
- soft drinks, colas, root and birch beers

Food products.
- baked beans
- biscuits, bread, brown bread
- butter
- canned meat products
- canned mushrooms and other vegetables
- catsup (tomato)
- cheezes
- condiments
- confections, caramels, icings
- fish pastes
- gravies and sauces
- ice cream and sherbet mixes
- jams, jellies, marmalades
- mince meat
- pet foods
- pudding mixes
- pickles and relishes
- soups
- table syrups
- vinegars

Alcoholic beverages.
- aperitifs, beer, brandy, cordials, liqueurs, rum, whiskey, wines

Caramel colors are used most extensively in soft drinks. In the United States, about 75 percent of the annual production is used in soft drinks. Information concerning the distribution of these colors in other products is not available.

B. Consumption of caramel colors.

It is estimated that about 70,000 tons of commercial caramel colors are produced annually in the United States. This suggests an average total consumption of approximately 0.85 grams per person per day.

in caramel-containing soft drinks, the amount of single strength color used is less than 4 grams per liter. Only one-half of this amount of double strength color is needed. Information about soft drink consumption indicates that consumption of more than one liter per day by any person would be considered unusual. In a normal serving, there is present only one gram or less of color. At this level of use, typical caramels (20,000 EBC) would contribute only approximately 60 parts per million of either combined nitrogen or sulfur.

C. The safety of caramel colors.

Many of the animal tests demonstrated that amounts of caramel equivalent to 700 grams per day could be ingested by a 70 kilogram man without effect. This amount is more than 800 times the estimated average total daily intake and approximately 700 times more than what is present in one serving of soft drink. One study with adult rats showed no effect at caramel ingestion levels two times higher. It is apparent that the present usage of caramel colors in food is very low. Its usage is certainly controlled not by toxicity considerations but by technical need in accordance with good manufacturing practice.

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The Toxicology and Chemistry of Ammoniated Feed Supplements

Recently, there has been some discussion of several isolated cases of hysteria and convulsions in beef cattle and other farm animals caused by the feeding of products prepared by ammoniation of sugar-containing agricultural wastes such as molasses. In addition, a toxic material was extracted from ammoniated molasses. A chemical, 4-methylimidazole, having convulsant properties has been isolated from ammoniated molasses extracts.

These observations have stimulated speculation that caramel colors that are prepared also by ammoniation, sometimes in the presence of sulfur dioxide, of starch hydrolysates, invert cane sugar or glucose, may also contain harmful chemicals. Because the results of many toxicological tests of caramel colors have been completely negative, the presence of 4-methylimidazole, or any other toxic chemical, in them had never been anticipated.

In view of a continuing need for reassurance that these colors are safe, it is appropriate to examine, firstly, the recorded history of observations relating to the use of ammoniated feed supplements, and of ammoniated molasses in particular, in animal diets; secondly, the pertinent aspects of ammoniated feed supplement chemistry; and, thirdly, the matter of similarity or lack of similarity between ammoniated molasses and commercial caramel colors.

1. Ammoniated feed supplements

The first references to ammoniated feed supplements are by Millar (1, 2, 3) during the years 1941-1944. He treated sugar beet pulp and corn silage with anhydrous ammonia gas at pressures of 10-200 pounds, at temperatures of 40-125°C and for times of 15-180 minutes. Products prepared from sugar beet pulp containing about 4 percent nitrogen were fed to cattle and sheep. All were readily accepted, whether fed alone or combined with other feed; only one (6.3 percent N) fed by itself was rejected, but

accepted if combined with other feed. Year old dairy stock ate liberal quantities of a product containing 4.4 percent nitrogen. No adverse reactions were observed in any of the feeding experiments.

During the next decade, a number of studies either carried out by the Commercial Solvents Corporation or supported by the Corporation on the preparation of ammoniated feed supplements from a variety of sugar wastes were reported (4, 5, 6, 7, 8, 9). Some of the materials used were cane or blackstrap molasses, hydrol syrup, condensed distillers molasses solubles, inverted molasses, wood sugar molasses, citrus molasses and citrus pulp. These materials were treated with anhydrous or liquid ammonia at temperatures from 20-150°C and for times of 2-12 hours; when required, suitable pressures were employed. Additional variation was introduced by neutralizing excess alkalinity with hydrochloric, sulfuric or phosphoric acids. The nitrogen content of these products attained 6.5 percent but was usually about 4.5 percent. None of these products was fed alone, but at levels no greater than 12 percent in a mixed ration. Bull calves fed six pounds of ration daily for 30 days grew and gained weight satisfactorily. No adverse effects of any kind were observed. In other experiments with dairy calves, milk cows, heifers, ammoniated molasses was fed at a level of 10 percent of the ration for 90 days. No ill effects were observed and no differences in metabolic parameters detected.

Research along similar lines at other locations (10, 11, 12, 13) yielded similar results. Ammoniated cane molasses could replace one-half of the linseed meal used as the protein source in a ration for beef cattle and give better performance with respect to protein and energy than linseed meal alone. Beef cattle fed ammoniated condensed distillers molasses solubles at levels from 5-10 percent of the ration showed no difference in growth from controls. Sheep fed a ration containing 10 percent of ammoniated cane molasses or ammoniated condensed distilled molasses solubles over a period of 225 days grew as well as the controls. No adverse effects were reported.

Beef cattle (251 in four experiments for 146 days) were fed several ammoniated products: molasses, citrus pulp and a saccharified by-product from furfural manufacture (14). At nitrogen levels equivalent to one-fifth or one-sixth of the protein in the rations, animals showed good efficiency of weight gain on all rations and a greater gain per day than controls when fed the ammoniated furfural by-product. No adverse effects were noted.

In a nitrogen balance study (15) cattle were fed a variety of ammoniated products prepared from molasses, residues derived from furfural manufacture, and dried distillers molasses solubles, in a mixed feed at levels ranging from 15 to 24 percent crude protein equivalent and compared with comparable rations containing soybean meal or urea as the major nitrogen source. After preconditioning, the animals were fed test rations for one week. In all cases, although the retention of consumed nitrogen by test animals was not as great as that by animals fed non-ammoniated products, their retention of absorbed nitrogen was quite comparable. No adverse effects were noted.

Ammoniated hydrol (corn molasses) was used as a protein substitute in a ration for wintering young beef cattle (16). It replaced one-half of the soybean meal component at a level of 1.2 percent crude protein equivalent. The test lasted 140 days. Animals fed the ammoniated product gained only 10 percent less per day than the control animals. No adverse effects were noted.

Many experiments in which a great variety of ammoniated carbohydrate-containing products were fed to farm animals have been carried out in the United States without any incidence of adverse effects. Only one report of adverse effects observed in American studies has been found (17). In this case, ammoniated molasses fed to beef cattle at levels of crude protein equivalent to 6 and 18 percent in a mixed feed ration gave not only poor growth but caused some animals in all groups to act in a crazy manner after having been on the ration for eight days. After transferring the animals to control diets, they recovered. When returned to the test diets, about one-third of the animals again were affected. No explanation for this effect was offered. The effect did not seem to be related to the extent of ammoniation or the amount of product fed. It is interesting to note, however, that this effect was not observed in a later experiment (16) described above in which ammoniated corn molasses was fed for 140 days to young beef cattle at a 1.2 percent level of crude protein equivalent.

Information received recently suggests that industrial efforts to prepare commercial ammoniated feeds for farm animals were discontinued for a number of reasons, one of which was related to the excitement factor. It was not possible to prevent farmers from feeding excessive amounts of these low cost feed supplements.

The first report from outside the United States of adverse effects in a feeding test of ammoniated sugar products was published by Wiggins in 1956 (18). He said

that some animals show a marked reaction to ammoniated invert molasses and mentioned, for example, one trial in which two steers were each fed one pound of ammoniated invert molasses (4 percent nitrogen) and three pounds of citrus pulp. Both took three days to consume this. Then, on being fed another portion of the feed, they consumed it more rapidly during the morning of the fourth day. In the afternoon, one animal developed a violent hysteria; the other animal was not affected at all. He said that similar experiences had been recorded in trials in England and that large scale use of ammoniated invert molasses in cattle feeds would be ruled out unless an answer could be found to the toxicity problem.

Wiggins (18) also reported that acidification of the ammoniated molasses with acid, such as phosphoric, acetic or lactic would render the product non-toxic. This was substantiated by a trial with sheep which were fed as much as two pounds of ammoniated product per day in a concentrated ration in two eight week periods separated by a three week rest period. The sheep were maintained in excellent condition and no adverse effects were observed. He also says in another report (19) that ammoniated molasses at one-half pound per day in a ration fed to water buffalo was palatable and showed no toxicity. On the other hand, one Zebu calf fed in the same manner suffered a severe bout of hysteria. Acidified product gave no indication of toxic disorders in sheep and cattle. In 1958, a patent was issued to Wiggins (20) indicating that acidified ammoniated molasses products were suitable for use as feed for ruminant animals.

The English studies referred to above were not published until 1958 (21). Tests were run on a British ammoniated invert molasses and on two similar products made in the British West Indies which had been treated with phosphoric or acetic acid in an attempt to render them non-toxic. In one cattle test, the level of British product in the ration was gradually increased to one pound (in 13 of ration). After two days at this level, three of five animals in this test group developed violent hysteria and blindness. They were given treatment and recovered. The experiment was abandoned. In another cattle test with the West Indies products, the animals receiving phosphoric acid treated product showed symptoms at a one-half pound level in the ration. After treatment and recovery, these animals were fed acetic acid neutralized product with no ill effects for 18 days. Animals started on acetic acid neutralized product showed no ill effects after 33 days.

Additional tests on weanling rats fed ammoniated molasses at levels increasing to 50 percent in a stock diet showed poor growth but no toxicity. Acetic acid treated product was toxic to guinea pigs when fed at levels higher than 10 percent in the diet.

Although the results of experiments with ammoniated feed supplements are conflicting in many instances, there is no doubt that these products can, under certain circumstances, produce undesirable reactions in some animals. Precisely what causes these reactions and why some animals react so violently when others do not, is not known. The relationship between chemical and toxicological information, as it exists at the present time, is described briefly in the following section.

2. The chemistry and toxicology of ammoniated sugar products

Wiggins (22) in a study of chemical changes occurring during ammoniation of molasses found by chromatographic analysis that a number of imidazole and pyrazine derivatives had been formed. A definite identification of 4(5)-methylimidazole was made. The list of identified compounds was extended (18) to include some pyrazines. By crude technics, he concluded that ammoniated molasses contained about 10 percent imidazoles and 20 percent pyrazines, and accounted for most of the fixed nitrogen.

In addition, in search of the cause of toxicity, Wiggins (18) made an ethyl acetate extract of ammoniated molasses, evaporated off the solvent, dissolved the residue in water to a level of 5.4 percent nitrogen and mixed this solution with chick starter ration in a ratio of 25 milliliters of solution to one pound of ration. He also prepared a ration made from the aqueous residue remaining after ethyl acetate extraction and starter in the same proportions. Chicks fed ethyl acetate extractables developed hysteria within three days; some died. Those fed the other fraction lived normally for 12 weeks. It seems clear that the toxic principles were extracted with ethyl acetate. Wiggins did not continue his work to associate toxicity with either imidazoles or pyrazines.

Recently "in view of the current interest in the safety of ammoniated commodities, including the use of ammonia in the deallergenization of castor bean pomace and for the detoxification of mycotoxin-contaminated oil seed meals," Nishie and her co-workers (23, 24) decided to reopen the investigation on the toxicity of some of

the compounds formed by interaction of ammonia with reducing sugars. A great variety of imidazoles and pyrazines were tested. Only 4-methylimidazole had any effect; it was found to be a convulsant in moderate doses ($CD_{50} \pm SE$[mg/kg], 360 ± 18, oral) in a decreasing order of sensitivity in rabbits, mice and chicks. At subconvulsant doses, spontaneous motor activity was also decreased in mice. The present state of knowledge indicates that 4-methylimidazole is the only toxic compound in ammoniated feed supplements.

About the pyrazines, Nishie et al. say they "are central depressants with weak hypnotic and anticonvulsant characteristics and possess a low order of toxicity. They can prevent seizures caused by 4-methylimidazole.

3. The relationship between caramel colors and ammoniated feeds

There is some similarity between the process used to combine ammonia with agricultural sugar containing wastes and that used to combine ammonia and corn syrup solids or invert sugar and also that used to combine ammonia, sulfur dioxide (sulfite) with corn syrup solids or invert sugar. There is obviously some difference in the composition of the end products in every case, inasmuch as the toxic effects observed after feeding ammoniated feed supplements to farm animals have never been observed after feeding the two major types of caramel to rats and dogs. This could be due to the following: (1) relatively complex mixtures of natural components in agricultural wastes are used in ammoniated feed supplement manufacture; (2) pure simple carbohydrates are used in caramel color manufacture; (3) these supplements are manufactured under alkaline conditions; caramel colors, under neutral to acid conditions; (4) supplements, especially those from ammoniated molasses, contain large amounts of imidazoles and pyrazines; and (5) caramel colors apparently do not contain these chemicals in any significant amounts. Unpublished analytical information recently obtained shows that caramels contain much less nitrogen and only from 0.002 - 0.020 percent of 4-methylimidazole. Work in progress will provide additional information on the 4-methylimidazole content of a variety of caramels.

Furthermore, levels of feeding ammoniated products in the diets of animals, or in human beings, provide some basis for comparing their toxic potentials. It is evident that about one pound of ammoniated feed supplement (4-6 percent nitrogen) at a level of 6-25 percent in a ration will cause hysteria in some farm animals. There has been a great variability in effect which may be related to differences

in composition of materials used for the preparation of ammoniated products. Caramels, on the other hand, which have much lower nitrogen contents and contain only traces of 4-methylimidazole, have been fed to animals at high levels, representing in man daily intakes of more than one pound, with no adverse effect. Furthermore, a high level of beverage consumption (five bottles per day) even by a child would represent ingestion of only one-sixtieth as much caramel color per kilogram of body weight on a nitrogen basis as compared with ammoniated molasses fed to cattle referred to above. Calculations, based on the Nishie studies of convulsive activity of 4-methylimidazole, indicate that a 20 kilogram child would have to drink about 30,000 ten-ounce bottles of beverage in one day to ingest a quantity of this imidazole equivalent to the CD_{50} value.

At this time, the facts permit the following conclusions: (1) Some ammoniated feed supplements, but not all, are toxic to some animals. (2) Several imidazoles and pyrazines have been isolated only from ammoniated molasses. (3) Only one of these compounds, 4-methylimidazole, is a toxicological convulsant. (4) Pyrazines are anti-convulsants. (5) Effects in some feeding experiments may be influenced by the relative proportions of 4-methylimidazole and pyrazines in different supplements. (6) No toxicity has ever been observed during experiments in which a variety of commercial caramel colors have been fed at high levels to rats and dogs. (7) Preliminary analyses indicate that insignificant amounts of 4-methylimidazole are produced during the preparation of commercial caramel colors. And (8), research on the chemical composition of caramel colors, particulary with reference to 4-methylimidazole, should continue.

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From The Research-Work on Pathological Physiology at Berlin University
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The Influence of Caramel on the Human Metabolism

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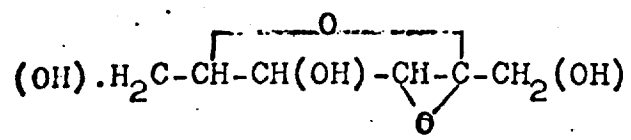
The Influence of Caramel on the Human Metabolism

For a long time, a lavish use has been made in public nourishment of foodstuffs and for luxury products that have been exposed to the roasting process. One of these is caramel which is a roasted product of sugar. Caramel has been figuring rather largely in public nourishment for quite a while. Food chemistry and also theoretical chemistry are interested in the composition of caramel.

The knowledge of the chemical nature of the products developing from the various sugars in the course of the roasting process has been much widened by the researches of Picted and his co-authors (2). They heated sugar in the vacuum at a temperature of 150°. In doing so they obtained crystalline bodies, chemically to be looked upon as sugar anhydrides. These sugar anhydrides are of the general composition Cn (H_2O)n-I! When heated, water is split off. This splitting off takes place between the group O-C-OH, the seat of the reducing property, and one of the alcoholic groups H-C-OH. Thus, glycosane is obtained from grape, sugar, levolusane from fruit-sugar. In the course of this process, cane-sugar is converted into a mixture of glycosane and levolusane. It is believed that also the parent substances of the disposable constituents of caramel are sugar anhydrides of the type of glucosane and levolusane.

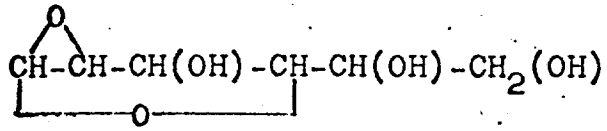
Just recently, some informing papers on caramel chemistry have been published by the German Scientific Research Institute for Food Chemistry in Munich and by the Carnegie-Institute in Pittsburg.

When cane-sugar is heated a dissociation in glycose and an anhydride of the fructose - levolusane - materializes that was already observed by Gélis. Upon a further rise of temperature to 185° up to 190°, one obtains a mixture of glycisane and levolusane that interact to form isosucrosane of the formula $C_{12}H_{20}O_{10}$. This process probably passes off as follows:



levolusane

+



glycosane

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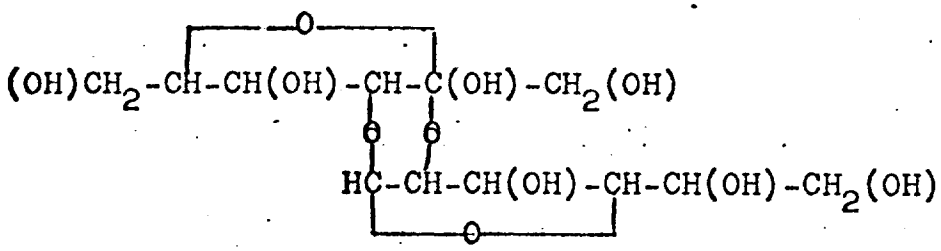

isosucrosane

Upon further heating, the caramellane and finally the caramellene of the formula $C_{36}H_{50}O_{25}$ was supposed to be formed. In the course of the latest researches it was found that the older views on the reactions taking place in the initial stages of the caramel formation are no longer sustainable. Kruisheer (3) made a very elaborate study of the question as to what changes in chemical respect result from the effect of high temperatures on sugar. Above all, he analyzed those substances that are formed upon roasting sugar, insulin and caramel formation. As regards the caramel formation, the result of his researches was that the views of Pictet and co-authors are no longer sustainable. He found that, when heated, part of the sucrose is converted into glycose and in an anhydride of the fructose - levolusane - but that another part is simultaneously converted into fructose and glycose anhydride. This process is observable by frothing since a loss of water takes place upon the formation of glycose anhydride. At the same time, there is also a formation of difructose and diglycose anhydride. He further found that the isosucrosane is no homogeneous body but a mixture of several substances among these probably also levolusin and glycosin. For this reason the existence of caramellane and finally of caramellene

must be considered to be unproved. According to the latest researches (Guenther von Elbe (4)) caramel is a colloidal dispersion of a lyophobic humic substance I in a mixture of 2 protective substnnces II and III.

Whereas the knowledge of the chemical structure is as yet incomplete, the use of caramel in public nourishment and in dietetic therapy has been a recognized procedure for a long while. It is used as sugar colour for dying beer, sauces, wines and for manufacturing sweets. In the medical profession it is used in treating diabetes.

As from 1851, observations have been on hand from Bouchardet (5) and Külz (5) from which it could be seen that not all kinds of carbohydrates act on the diabetically diseased organism in the same way. It was found that grape-sugar, maltase, and starch produce the quickest and strongest glycosuric effect, contrary to levolose and lactose that produce but half as strong an effect. Efforts were now made to find other carbohydrates of a less glycosuric effect for the diabetic to replace the main carbohydrates, cane-sugar and starch. They were called "substitute carbohydrates" because they are no part of the usual food. Among these are i.al. caramel, roasted starch preparations, and anhydro sugar.

In 1914, Grafe (6) was the first to use caramel in treating diabetes. In doing so, he proceeded from the idea to release the sugar molecule, by even slight transformations therein, from its glycosuric effect in order to make it suitable for the diabetic diet. He heated dry glycose up to its melting point at 146°. The sugar thereby loses its ability to be fermented by the yeast and its sweet flavour. A blackish brown product has developed, caramel. Grafe now tested the caramel for its suitability for the diabetic. He found the caramel to have a favourable effect on the glycosuria. A good preparation, Merck's "Karamose", was introduced in the therapy. Because of its too high production costs, this preparation no longer exists today having been very popular with all diabetics thanks to its pleasant flavour. 50 up to 100 g of caramel are well tolerated without ill effects by all diabetics; whereas Umber (7) and von Noorden (8) warn against administering larger doses of caramel without first testing the effect on the glycosuria. Larger quantities

of caramel also often provoke diarrhoeae and thus cause a poorer resorption. As described in detail in the experimental part herein after, 120 g of caramel were tolerated without ill effects by myself. Grafe advises to administer opium on caramel days. Even in case of a normal resorption, as much as 5 up to 30 per cent of the caramel consumed are excreted faeces unutilized and are thus lost to the metabolism. Von Noorden (8) suggests to take the preparation but twice a week since, with some diabetics who had been completely without sugar for months, glycosuria redeveloped following caramel doses. For pleasanter use Umber (7) gives a few recipes. Delicious meringues, enjoyable with coffee or tea, are made like this: 300 g of whipped whites are mixed with fine ground vanilla and 200 g of caramel, baked in the form of small biscuits at medium heat for one hour, and, if required, spread with a solution of drystallose. Another sweet, caramel cream, is cooked as follows: 50 g of caramel are boiled up together with 200 g of cream and some vanilla, then whipped with 2 yolks, then reduced to a cream-like compactness on a hot water bath and finally dashed with a few drops of a 20 per cent crystal saccharine solution to suit the individual taste.

The caramel therapy showed a good effect on the glycosuria. But there was also a favourable effect on the acidosis. Furthermore, the respiratory quotient went up. For this reason it is advisable to administer caramel especially in such cases as are liable to acidosis.

The above clearly shows that carbohydrate substitutes, among these also caramel, may find useful application with light and medium severe cases of diabetes. The carbohydrate substitutes may be administered as saccharin or calory dispenser depending on the condition of the case or the type of preparation. One gram of caramel has a caloric value of 4.4 calories. By adding caramel to the diet, substantial quantities of calories (200 up to 400 calories per day) may thus be administered to the often seriously underfed organism even though not all caramel consumed is resorbed. Moreover, the carbohydrate substitutes, being derivates of sugar, develop all the physiologic effects as produced during the splitting of the natural carbohydrates. It is believed that they need not first be converted into grape-sugar in the organism but that the

diabetic body can convert them directly into glycogen which may help to save insulin.

The above exposition on caramel gave us an insight into the metabolic process of the organism after the administration of caramel. It now devolved upon me to test in a self-experiment the effects on the oxidative metabolic condition of the whole organism according to the behaviour of the urine quotients C:N and vacat-O:N upon administration of caramel in the food (9). Said quotients relate to the ratio of the quantities of carbon excreted in the urine ot the quantities of nitrogen (carbon quotient C/N) and of such quantity of oxygen, as must be conveyed to the urine in order to effect a complete oxidation of all its dissolved components, to the quantities of nitrogen excreted in the same urine (oxidation quotient vacat-O:N). The state of the urine quotients thus provides a new source of information about the metabolic process. It shows us a cross-section through the total metabolism that is on a different level than the basal metabolism. This method has already been used to define the exidative state in the intermediate metabolism upon the administration of roasted products (10).

After Valentin (II) and Heusch (12) in their experiments with roasted wheaten flour or rye flour respectively and Traute (13) in his experiments with bread-crust as compared with bread-crumbs established a decreased oxidative state in the intermediate metabolism and the almost negative result of the meat test by Traute (13) had lead to the conclusion that it could not be the roasted albumin that produced these pronounced changes, Schering (14) endeavoured to bring about a final decision. The experiments with roasted casein by Schröder (15) and those of Kuhn's (16) with roasted wheat gluten albumin had also shown that the roasted products of pure albumin did not produce a substantial change in the state of the urine quotients. Schering (14) now tested the influence of the consumed caramelized beetroot-sugar on the oxidative situation in the intermediate metabolism. In his rat experiments he found that, upon the administration of caramelized beetroot-sugar, a distinct increase of the urine quotients developed which was probably conditioned by an adaptation in the intermediate metabolism.

It now devolved upon me to check the findings of Schering's (14)

by means of a self-experiment in order to find whether said metabolic changes are also produced by the caramel doses customary in the human therapy. In doing so I adhered, on principle, to the experimental procedure applied by Traute (13) Valentin (11) Heusch (12) Schröder (15) Kuhn (16) and Schering (14) in their rat experiments. For 20 days I lived on a diet with an albumin-content of approx. 56 g (= 9,o4 g of N) and a caloric content of approx. 2300, my body weight being 85 kg. The computation of the albumin-content and caloric content took place according to the Schall's tables (17).

	K.H.	quantity	albumin	cal.
lean beef, cooked	0,0	100	30,0	300
white wheaten bread	51,0	100	6,8	270
rice, polished	78,0	100	7,9	356
milk	10,0	200	6,8	134
sugar (beetroot-sugar)	100,0	100	-	410
tomatoes	4,0	100	1,0	33
apples	26,0	200	0,8	116
salade, green	4,0	200	2,8	32
oil	0,0	20	-	180
lemon	0,0	-	-	-
butter	0,0	60	0,4	471
	273,0		56,5	2302

During the entire period of the experiment, while performing my duties as hospital intern as usual, my weight remained practically constant. In my first experiment, following a preparatory period of 2 days on which the test diet was consumed but no urine-analyses were made, said diet was adhered to over a preliminary period of 5 days. Subsequently, the sugar in the diet was replaced by caramel in quantities corresponding to the substituted sugar. The caramel was obtained by roasting from the same supply of beetroot-sugar that also provided my diet with sugar. At first, I had 60 g of caramel for 3 days, then 90 g of caramel on the 9th and 10th day and finally 120 g of caramel, the quantity equivalent to 100 g of beetroot-sugar, which I tolerated without bad effects. Contrary to the tests of Schering's who had established a pronounced increase

of the urine-quotients in his rats, it was found that said increase of the urine-quotients at first failed to show up in my experiment. Only upon large doses of 120 g of caramel, a trifling upward movement of the urine-quotients, particularly of the carbon-quotients, could perhaps be observed if compared to the individual daily figures. In a second test directly following the first, I first once again lived on a caramel-free diet, that is to say for 4 days. Subsequently, 100 g of sugar of said diet were immediately substituted by the equivalent quantity of 120 g of caramel and this large dose of caramel was consumed for 3 days. The result was the same as in the first test. My diet contained approx. 270 g of carbohydrates of which 100 g were beetroot-sugar. On the days when I had the largest quantity of caramel upon complete substitution fo said quantity of sugar, far more than one third of the total carbohydrate-content of the food had been replaced by caramel.

My two experiments, in connection with the experiments and observations of Schering's show that caramel can only take effect on the oxidative condition in the intermediate metabolism if taken in larger quantities, reckoned per kg of body weight. For in Schering's rat experiments, approx. 14.0 g of caramel were administered per 1 kg of body weight whereas I consumed only 1.4 g of caramel per kg of body weight. With Schering's rats, the absolute C and vacat-O factors were higher in all caramel periods as contrasted with the caramel-free experiments. But the urine-N reacted differently in the two experiments: with Schering it went up during the caramel period in one experiment, in the other it went down during this period. In my self-experiment, the urine-N factors were lower in the caramel periods when compared with the corresponding periods without caramel doses. Reckoning the daily food to contain 9,3 g of N, a rough calculation shows that with periodic average values of 9,3, 8,1, 8,2, 7,6 g of urine-N these probably was an N-equilibrium..Most likely, the caramel excretion in the faeces increased as with larger doses of caramel and such was the indirect cause of larger N-losses through the intestinal canal. For we know that, if larger doses of caramel are administered, the human bowels excrete caramel to a considerable extent unchanged. However, with Schering's rats, neither the quantity of dry faeces nor the quantity of faeces-N was increased during the caramel periods as compared to the other periods. During the caramel period

of the test, the absolut values for C and vacat-O went down wellnigh conforming to the N-values. During the caramel period of the second test, the change of the values for C and vacat-O followed that of the values for N perhaps a little more hesitantly.

In view of the dietetic therapy in cases of diabetes, it is nevertheless important to the hospital to know that caramel in the quantities as used there produces no effect worth mentioning on the oxidative condition. In the light of Schering's rat-experiments it seems doubtful that the reduction of the urine/N factors as found in my experiments is obligatory for the administration of caramel.

Experimental Part

The test was made on myself in two series of experiments and took 20 days. Following a preparatory period of 2 days, the analyses commenced on the 3rd day. During the first 4 days of each period I had a diet with 100 g of beetroot-sugar. Whereas, in the first series, I steadily increased the caramel doses every other day from at first 60 g, then 90 g and finally 120 g, I consumed 120 g of caramel right away in the second test series. The 24 hours' urine quantity was collected, measured and a sample of same was analysed.

My diet was composed as follows:

Periods 1 and 3

lean beef, cooked	100 g
white wheaten bread	100 g
polished rice	100 g
milk	200 ccm
sugar (beetroot-sugar)	100 g
fresh tomatoes	100 g
fresh apples	200 g
green salad	200 g
oil	20 g
butter	60 g

In the second period I consumed a diet with 50 % of the sugar in the form of caramel (60 g) on the 7th and 8th day, 75 % of the sugar were substituted on the 9th and 10th day by 90 g of caramel and on the 11th and 12th day I substituted 100 g of sugar by 120 g of caramel = 100 %. In the 4th period, from the 16th up to the 18th day of the analyses, I consumed 120 g of caramel right away.

In the course of the whole day my meals were distributed as follows:

Breakfast:	30 g of white bread and 30 g of butter, tea unrestricted.
Luncheon:	100 g of lean cooked beef, 40 g of white bread, salad of 200 g of green salad and 100 g of tomatoes, 20 g of oil and 50 g of sugar.
Dinner:	100 g of polished rice boiled with 200 g of milk and 50 g of sugar. Also 30 g of white bread and 30 g of butter.

On the caramel days I consumed caramel in tea distributed over the various meals. I also had 200 g of fresh raw apples and 2 lemons per day.
I drank as much tea as I wanted.

Analytical Part

The urine was collected and its quantity measured in 24 hours. In the 24 hours' quantity of urine were determined: the nitrogen according to the half micro method of Kjedahl, the carbon according to the micro method of Nicloux-Osuka (18), the required oxygen according to the micro method of Müller-Kanitz (19).

Day of experiment	body weight	quantity of urine	Urine C mg.	Urine vacat-O mg.	Urine N mg.	C/N	V.O/N	V.O/C
			1st Period - Sugar					
1	85,0	920	7419	11408	7972	0.93	143	1.54
2	85.0	735	8110	12661	9364	0.87	1.34	1.56
3	84.9	750	8285	12893	9461	0.80	1.35	1.56
4	84.9	1400	8434	12020	9996	0.84	1.20	1.42
5	84.9	1080	8729	13549	9540	0.91	1.42	1.55
Periodic average figures (Division of the average figures for C, VO and N gives the average figures of the quotients.)			8195	12506	9267	0.88	1.35	1.53
			2nd Period - Caramel					
6	84.9	1200	7187	10543	7845	0.91	1.34	1.47
7	84.9	750	8303	11700	9576	0.87	1.22	1.41
8	84.9	1175	7776	11413	8948	0.87	1.28	1.47
9	84.8	650	5831	11500	7116	0.82	1.62	1.98
10	84.7	985	7446	12676	8274	0.90	1.46	1.61
11	84.7	1060	7333	11235	6752	1.09	1.66	1.53
Periodic average figures			7309	11396	8085	0.90	1.41	1.56
			3rd Period - Sugar					
12	84.8	930	6891	10888	7708	0.89	1.41	1.58
13	84.7	600	7031	11160	7694	0.93	1.45	1.59
14	84.7	530	7266	11116	7806	0.93	1.42	1.53
15	84.6	910	8097	12791	9708	0.83	1.32	1.58
Periodic average figures			7321	11488	8228	0.89	1.40	1.57
			4th Period - Caramel					
16	84.6	960	7402	11863	7795	0.95	1.52	1.60
17	84.7	1370	6220	10485	7069	0.88	1.48	1.69
18	84.6	1400	7787	11946	7987	0.97	1.49	1.53
Periodic average figures			7136	11431	7617	0.94	1.50	1.60

Synoptical Table

Period No.	Body weight in kg	Urine-C mg	Urine vacat-O mg	Urine-N mg	C/N	V-O/N
I. 100 g Sugar	85.0	8195	12506	9266	0.88	1.34
II. Caramel!	84.7	7309	11396	8085	0.90	1.40
III. Sugar	84.8	7321	11488	8228	0.89	1.40
IV. Caramel!	84.6	7136	11431	7617	0.94	1.60

Aus den seminaristischen Uebungen für pathologische Physiologie
an der Universität Berlin.
(Leiter: Prof. Dr. Adolf Bickel)

Ueber den Einfluß des Karamels auf den menschlichen Stoffwechsel

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Inaugural-Dissertation

zur

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Ueber den Einfluß des Karamels auf den menschlichen Stoffwechsel

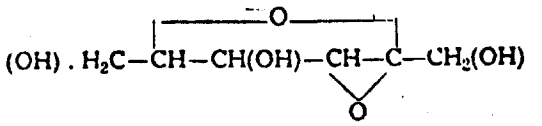
Im Rahmen der Volksernährung finden seit langem Nahrungs- und Genußmittel ausgiebige Verwendung, die dem Röstprozeß unterworfen worden sind. Zu Ihnen gehört auch der Karamel, der ein Röstprodukt des Zuckers ist. Karamel spielt schon seit langer Zeit bei der Volksernährung eine nicht unerhebliche Rolle. Die Nahrungsmittelchemie und auch die theoretische Chemie sind an der Zusammensetzung des Karamels interessiert.

Die Kenntnis der chemischen Natur der Produkte, die beim Röstvorgang aus den verschiedenen Zuckern entstehen, ist durch die älteren Arbeiten von Gélis (1) vor allem aber durch die Untersuchungen von Pictet und seinen Mitarbeitern (2) weitgehend aufgeklärt worden. Sie erhitzten Zucker im Vakuum bei einer Temperatur von 150°. Hierbei erhielten sie kristallinische Körper, die chemisch als Zuckeranhydride anzusehen waren. Diese Zuckeranhydride haben die allgemeine Zusammensetzung $Cn(H_2O)n-1$! Bei der Erhitzung wird Wasser abgespalten. Diese Abspaltung findet statt zwischen der Gruppe O—C—OH, dem Sitz der reduzierenden Eigenschaft, und einer der alkoholischen Gruppen H—C—OH. Es entsteht so aus dem Traubenzucker Glukosan, aus Fruchtzucker Lävulosan. Rohrzucker geht bei diesem Prozeß in ein Gemisch von Glucosan und Lävulosan über. Es wird angenommen, daß auch die Grundkörper der ausnutzbaren Bestandteile des Karamels Zuckeranhydride nach der Art des Glucosans und des Lävulosans sind.

In letzter Zeit sind einige aufschlußreiche Arbeiten zur Karamelchemie aus der deutschen Forschungsanstalt für Nahrungsmittelchemie in München und aus dem Carnegie-Institut in Pittsburg erschienen.

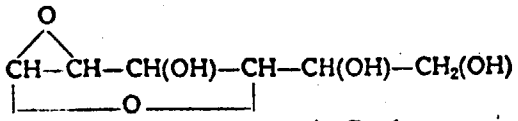
Erhitzt man Rohrzucker so tritt ein Zerfall in Glykose und ein Anhydrid der Fruktose — Lävulosan — ein, den schon Gélis beobachtete. Bei weiterer Temperatursteigerung auf 185° bis 190° erhält man eine Mischung aus

Glykosan und Lävulosan, die aufeinander einwirken, um Isosaccharosan zu bilden von der Formel $C_{12}H_{20}O_{10}$. Dieser Vorgang geht wahrscheinlich folgendermaßen vonstatten:



Lävulosan

+



Glukosan

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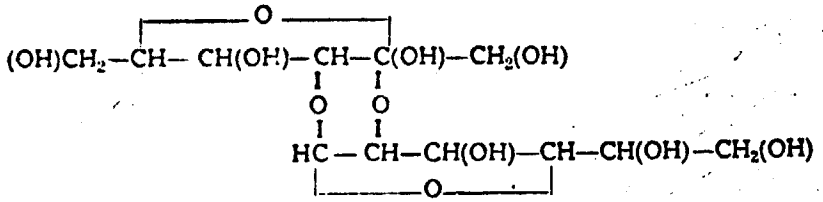

Isosaccharosan.

Bei weiterem Erhitzen sollte das Karamelan und schließlich das Karamelen von der Formel $C_{36}H_{50}O_{25}$ entstehen. Bei den neueren Untersuchungen stellte sich heraus, daß die älteren Anschauungen über die Umsetzungen, die in den ersten Stadien der Karamelbildung vonstatten gehen, nicht mehr haltbar sind. Kruisheer (3) arbeitete sehr ausführlich über die Frage welche Veränderungen in chemischer Hinsicht bei der Einwirkung hoher Temperaturen auf Zucker entstehen. Er untersuchte vor allem die Stoffe, die bei der Röstung der Zucker entstehen, das Insulin und die Karamelbildung. Bei der Karamelbildung kam er zu dem Ergebnis, daß die Auffassungen Pictets und seiner Mitarbeiter nicht mehr haltbar sind. Er fand, daß ein Teil

der Saccharose beim Erhitzen in Glykose und in ein Anhydrid der Fruktose — Lävulosan —, ein anderer Teil aber gleichzeitig in Fruktose und Glykoseanhydrid übergeht. Dieser Prozeß macht sich durch Aufschäumen bemerkbar, da ein Wasserverlust auftritt bei der Bildung des Glykoseanhydrids. Gleichzeitig entstehen noch Difruktose- und Diglykoseanhydride. Er fand weiter, daß das Isosaccharosan kein einheitlicher Körper ist, sondern ein Gemisch mehrerer Stoffe, darunter wahrscheinlich auch Lävulosin und Glykosin. Aus diesem Grunde ist auch das Bestehen des Karamelans und schließlich des Karamelens als nicht erwiesen zu betrachten. Nach den neuesten Forschungen (Guenther von Elbe (4)) ist Karamel eine kolloidale Dispersion einer lyophoben Huminsubstanz I in einem Gemisch von 2 Schutzsubstanzen II und III.

Während die Erkennung der chemischen Struktur noch nicht abgeschlossen ist, steht die Verwendung des Karamels in der Volksernährung und in der diätetischen Therapie schon seit langem fest. Er wird als Zuckercouleur zum Färben von Bier, Saucen, Weinen und zur Herstellung von Bonbons verwandt. In der Medizin findet er bei der Behandlung des Diabetes Anwendung.

Seit dem Jahre 1851 liegen von Bouchardet (5) und Külz (5) Beobachtungen vor, aus denen sich ergab, daß nicht alle Arten von Kohlehydraten in gleicher Weise auf den diabetisch erkrankten Organismus wirken. Man fand, daß Traubenzucker, Maltase und Stärke am schnellsten und stärksten glykosurisch wirken, im Gegensatz zur Lävulose und zum Milchzucker, die nur halb so stark wirken. Man bemühte sich nun an Stelle der Hauptkohlehydrate, Rohrzucker und Stärke, für den Diabetiker andere Kohlehydrate ausfindig zu machen, die weniger glykosurisch wirken als diese. Sie wurden „Ersatzkohlehydrate" genannt, da sie außerhalb der gewöhnlichen Kost gelegen sind. Zu ihnen gehören unter anderen der Karamel, geröstete Stärkepräparate und die Anhydrozucker.

Karamel wurde 1914 von Grafe (6) in die Behandlung des Diabetes eingeführt. Er ging dabei von dem Gedanken aus, durch schon geringfügige Umwandlungen im Zuckermolekül, dieses seiner glykosurischen Wirkung zu

entheben, um es für die Ernährung der Diabetiker nutzbar zu machen. Er erhitzte trockene Glukose bis zu ihrem Schmelzpunkt, der bei 146° liegt. Hierbei verliert der Zucker die Fähigkeit, durch Hefe vergoren zu werden und seinen süßen Geschmack. Es ist ein schwarzbraunes Produkt entstanden, der Karamel. Grafe prüfte jetzt den Karamel auf seine Verwendbarkeit beim Diabetiker. Hierbei stellte er eine günstige Wirkung des Karamels auf die Glykosurie fest. Ein gutes Präparat, die „Karamose" von Merck, wurde in die Therapie eingeführt. Wegen zu hoher Herstellungskosten gibt es heute dieses Präparat nicht mehr, das wegen seines angenehmen Geschmacks bei allen Diabetikern sich großer Beliebtheit erfreute. 50 bis 100 g Karamel werden von allen Diabetikern gut und reizlos vertragen; dagegen warnen Umber (7) und von Noorden (8) größere Dosen Karamel zu geben, ohne vorher die Wirkung auf die Glykosurie geprüft zu haben. Größere Mengen Karamel rufen auch oft Durchfälle hervor und bewirken somit eine schlechtere Resorption.. Von mir wurden, wie später im experimentellen Teil näher beschrieben wird, 120 g Karamel reizlos vertragen. Grafe empfiehlt an Karameltagen Opium zu geben.. Bei normaler Resorption werden immer noch 5 bis 30 % des genossenen Karamels unverbraucht im Kot wieder ausgeschieden, die somit für den Stoffwechsel verloren sind. Von Noorden (8) schlägt vor, das Präparat wöchentlich nur zweimal zu nehmen, da bei einigen Diabetikern, die monatelang völlig zuckerfrei waren, wieder nach Karamelgaben Glykosurie auftrat. Für die angenehmere Verwendung gibt Umber (7) einige küchentechnische Ratschläge. Ein sehr wohlschmeckendes Schaumgebäck, das zum Kaffee oder Tee gern genommen wird, läßt sich folgendermaßen herstellen: 300 Gramm Eiereiweiß werden zu sehr steifem Schnee geschlagen, mit feingestoßener Vanille und 200 Gramm Karamel vermengt, bei mäßiger Hitze eine Stunde in Form kleiner Plätzchen gebacken und dann eventuell mit Kristalloselösung bestrichen. Eine weitere Speise, der Karamelcreme, wird wie folgt bereitet: 50 Gramm Karamel werden mit 200 Gramm Sahne und etwas Vanille aufgekocht, dann mit 2 gut verrührten [illegible] durchgeht, dann auf dem Wasser[illegible]

cremeartiger Konsistenz eingeengt und schließlich je nach persönlichem Geschmack mit mehr oder weniger Tropfen einer 20 %igen Krystallsacharinlösung versetzt.

Bei der Therapie mit Karamel zeigte sich eine günstige Wirkung auf die Glykosurie. Es trat aber auch eine günstige Wirkung auf die Acidose ein. Ferner stieg der respiratorische Quotient. In einigen Fällen trat auch eine spezifisch-dynamische Wirkung auf. Ratsam ist es daher, Karamel besonders bei denjenigen Fällen zu geben, die zur Acidosis neigen.

Aus diesen Ausführungen geht zur Genüge hervor, daß die Ersatzkohlehydrate, zu denen auch der Karamel gehört, bei leichten und mittelschweren Fällen von Diabetes nutzbringend angewandt werden kann. Man kann die Ersatzkohlehydrate je nach Lage des Falles und je nach Art des Präparates entweder als Süßstoff oder als Kalorienspender geben. Ein Gramm Karamel zeigt einen Brennwert von 4,4 Kalorien. So kann man durch Zutat von Karamel zur Diät dem Organismus, der oft in seiner Ernährung stark heruntergekommen ist, erhebliche Kalorienmengen zuführen (200 bis 400 Kalorien täglich), auch wenn nicht alles genossene Karamel resorbiert wird. Außerdem entfalten die Ersatzkohlehydrate als Zuckerderivate alle die physiologischen Wirkungen, die den Umsatz der natürlichen Kohlehydrate brauchen, wie man annimmt, nicht erst im Organismus in Traubenzucker umgewandelt zu werden, sondern können wahrscheinlich vom diabetisch erkrankten Körper direkt in Glykogen übergeführt werden und wirken so vielleicht insulinsparend.

In den bisherigen Ausführungen über den Karamel erhielten wir bereits einen Einblick in das Stoffwechselgeschehen des Organismus nach Verabreichung von Karamel. Mir fiel nun die Aufgabe zu, in einem Eigenversuch die Auswirkungen auf die oxydative Stoffwechsellage des Gesamtorganismus nach Maßgabe des Verhaltens der Harnquotienten C : N und Vacat-O : N bei Verabreichung von Karamel in der Nahrung zu prüfen (9). Diese Quotienten betreffen die Verhältnisse der im Harn ausgeschiedenen Kohlenstoffmengen zu den Stickstoffmengen (Kohlenstoffquotient C/N) und derjenigen Menge Sauerstoffs, die man

dem Harn zuführen muß, um eine vollständige Oxydation seiner sämtlichen gelösten Bestandteile zu erzielen, zu den in denselben Harn ausgeschiedenen Stickstoffmengen (Oxydationsquotient = Vacat-O : N). Die Harnquotientenlage stellt so eine neue Orientierungsmöglichkeit über das Stoffwechselgeschehen dar. Sie zeigt uns einen Querschnitt durch den Gesamtstoffwechsel, der in einer anderen Ebene liegt als die des Grundumsatzes. Diese Methode ist auch bereits zu der Beurteilung der Oxydationslage im Zwischenstoffwechsel bei der Darreichung von Röstprodukten angewandt worden (10).

Nachdem Valentin (11) und Heusch (12) in ihren Experimenten mit geröstetem Weizen- beziehungsweise Roggenmehl und Traute (13) in seinen Versuchen mit Brotkruste im Vergleich zur Brotkrume eine herabgesetzte Oxydationslage im intermediären Stoffwechsel festgestellt hatten, und man durch den fast negativen Ausfall des Fleischversuches von Traute (13) zu dem Schluß gekommen war, daß es nicht das geröstete Eiweiß sein konnte, das diese starken Aenderungen herbeiführte, suchte Schering (14) eine endgültige Entscheidung herbeizuführen. Die Versuche mit geröstetem Kasein von Schröder (15) und die von Kuhn (16) mit geröstetem Weizenklebereiweiß hatten ebenfalls dargetan, daß die Röstprodukte des reinen Eiweißes die Harnquotientenlage nicht wesentlich verändern. Schering (14) prüfte nun den Einfluß des genossenen karamelisierten Rübenzucker auf die Oxydationslage im Zwischenstoffwechsel.. Er fand bei seinen Versuchen an Ratten, daß bei Verabreichung karamelisierten Rübenzuckers eine deutliche Steigerung der Harnquotienten auftrat, die wahrscheinlich durch eine Umstellung im intermediären Stoffwechsel bedingt war.

Mir fiel nun die Aufgabe zu, durch einen Selbstversuch die Untersuchungsergebnisse Scherings (14) nachzuprüfen, um festzustellen, ob diese Stoffwechseländerungen auch schon bei der Karameldosierung auftreten, die in der menschlichen Therapie üblich ist. Dabei hielt ich im Prinzip dieselbe Versuchsanordnung ein, die bereits Traute (13), Valentin (11), Heusch (12), Schröder (15), Kuhn (16), und Schering (14) angewandt hatten bei ihren Rattenversuchen. 20 Tage lang nahm ich eine Kost zu mir mit einem Eiweißgehalt von ca. 56 g (= 9,04 g N) und einem Gehalt an Kalorien von ca. 2300 bei einem Körpergewicht von 85 kg. Die Berechnung des Eiweißgehaltes und Kaloriengehalts erfolgte nach den Schallschen Tabellen (17).

Es enthielten:

	K. H.	Menge i. g.	Eiweiß i. g	Kal.
Mageres Rindfleisch gekocht	0,0	100	30,0	300
Weißes Weizenbrot	51,0	100	6,8	270
Reis (poliert)	78,0	100	7,9	356
Milch	10.0	200	6,8	134
Zucker (Rübenzucker)	100.0	100	—	410
Tomaten	4.0	100	1.0	33
Aepfel	26.0	200	0,8	116
Salat grün	4.0	200	2,8	32
Oel	0.0	20	—	180
Zitrone	0,0	—	—	—
Butter	0.0	60	0,4	471
	273,0		56,5	2302

Während der ganzen Versuchszeit, in der ich meiner Beschäftigung als Krankenhausarzt vollauf nachging, blieb das Gewicht annähernd konstant. In meinem ersten Versuche wurde nach einer Vorbereitungszeit von 2 Tagen, an denen die Versuchsdiät bereits genossen wurde, ohne daß aber Harnanalysen gemacht wurden, in einer ersten Periode von 5 Tagen diese Diät eingehalten. Darauf wurde Karamel in die Nahrung ersatzweise für den in ihr vorhandenen Zucker und zwar in den dem ersetzten Zucker entsprechenden Menge eingeführt. Der Karamel war durch Rösten aus demselben Vorrat von Rübenzucker genommen, aus dem auch meine Diät mit Zucker versorgt wurde. Anfangs nahm ich 3 Tage lang 60 g Karamel, dann am 9. und 10. Tage 90 g Karamel und schließlich am 11. Tage die 100 g Rübenzucker äquivalente Menge Karamel von 120 g, die ich reaktionslos vertrug. Es zeigte sich hierbei im Gegensatz zu den Untersuchungen Scherings, der eine kräftige Steigerung der Harnquotienten bei seinen Ratten festgestellt hatte daß diese Harnquotientensteigerung in

meinem Versuch zunächst ausblieb. Nur bei den großen Gaben von 120 g Karamel machte sich vielleicht eine Andeutung von einem In-die-Höhe-Drängen der Harnquotienten, besonders der Kohlenstoffquotienten, bemerkbar bei vergleichsweiser Betrachtung der einzelnen Tageswerte. In einem an den ersten Versuch angeschlossenen zweiten Versuch wurde zunächst wieder karamelfreie Kost und zwar 4 Tage lang genommen. Alsdann wurden sofort 100 g Zucker dieser Kost durch die diesem entsprechenden 120 g Karamel ersetzt und diese große Karameldosis 3 Tage lang genossen. Das Ergebnis war dasselbe wie im ersten Versuch. Meine Diät enthielt ca. 270 g Kohlehydrate, davon waren 100 g Rübenzucker. An den Tagen, an denen ich die größte Karamelmenge unter völligem Ersatz dieser Zuckermenge zu mir nahm, war also weit über ein Drittel des gesamten Kohlehydratgehaltes der Nahrung durch Karamel ersetzt.

Aus meinen beiden Versuchen geht in Verbindung mit den Versuchen und Beobachtungen Scherings hervor, daß Karamel nur in größeren Mengen, pro kg Körpergewicht gerechnet, einen Einfluß auf die Oxydationslage im intermediären Stoffwechsel ausüben kann. Kamen doch auf 1 kg Körpergewicht bei Ratten Scherings ca. 14,0 g Karamel, während bei mir auf das kg Körpergewicht nur etwa 1,4 g Karamel entfielen. Bei den Ratten Scherings waren in allen Karamelperioden die absoluten Werte für C und Vacat-O gegenüber den karamelfreien Versuchen erhöht. Der Harn N aber verhielt sich in beiden Versuchen verschieden; bei Schering: in dem einen stieg er in der Karamelperiode an, in dem anderen Versuch war er in dieser Periode erniedrigt. In meinem Selbstversuch waren in den Karamelperioden die Harn-N-Werte gegenüber den korrespondierenden Perioden ohne Karamelgaben erniedrigt. Eine Ueberschlagrechnung ergibt bei der Annahme von 9,3 g N in der täglichen Nahrung, daß bei den Periodenmittelwerten von 9,3 - 8,1 - 8,2 - 7,6 g Harn N wohl N Gleichgewicht bestanden haben dürfte. Es ist wahrscheinlich, daß mit der größeren Karamelmenge auch die Karamelausscheidung durch den Kot anstieg, und daß das indirekt zu größeren

N-Verlusten durch den Darm Veranlassung gab.. Wir wissen ja, daß der menschliche Darm bei größeren Karamelgaben Karamel in beachtlichem Umfange unverändert ausscheidet. Bei den Ratten Scherings war aber in den Karamelperioden weder die Trockenkotmenge, noch die Kot-N-Menge gegenüber den anderen Perioden erhöht. Die absoluten Werte für C und Vacat-O sanken in der Karamelperiode des Versuches annähernd konform mit den N-Werten. In der Karamelperiode des zweiten Versuches folgte die Aenderung der Werte für C und Vacat-O derjenigen der Werte für N vielleicht ein wenig zögernder.

Für die Klinik ist es im Hinblick auf die diätetische Therapie beim Diabetes immerhin von Bedeutung zu wissen, daß durch Karamel in den hier gebräuchlichen Umfange die Oxydationslage nicht nennenswert beeinflußt wird. Daß die in meinen Versuchen aufgetretene Reduktion der Harn-N-Werte für die Karamelgabe obligatorisch ist, scheint im Hinblick auf den Rattenversuch von Schering zweifelhaft.

Experimenteller Teil.

Der Versuch wurde in zwei Versuchsreihen an mir selbst durchgeführt und dauerte 20 Tage. Nach einer Vorbereitungszeit von 2 Tagen begannen am 3. Tage die Analysen. In den ersten 4 Tagen jeder Periode nahm ich eine Kost mit 100 g Rübenzucker zu mir.. Während ich in der ersten Reihe immer von 2 zu 2 Tagen die Karamelgaben von anfangs 60 g dann 90 g und schließlich auf 120 g steigerte, nahm ich in der zweiten Versuchsreihe sofort 120 g Karamel zu mir. Es wurde die 24-stündige Harnmenge gesammelt, gemessen und eine Probe hiervon analysiert.

Meine Kost hatte folgende Zusammensetzungen:

Periode 1 und 3

Mageres Rindfleisch gekocht	100 g
Weißes Weizenbrot	100 g
Polierter Reis	100 g
Milch	200 ccm
Zucker (Rübenzucker)	100 g
Frische Tomaten	100 g
Frische Aepfel	200 g
Grüner Salat	200 g
Oel	20 g
Butter	60 g

In der zweiten Periode nahm ich am 7. und 8. Tag eine Diät mit 50 % des Zuckers als Karamel (= 60 g) zu mir, am 9. und 10. Tage wurden 75 % des Zuckers ersetzt 90 g Karamel und am 11. und 12. Tage ersetzte ich 100 g Zucker durch 120 g Karamel = 100 %. In der 4. Periode nahm ich vom 16. bis 18. Tage der Analysen sofort 120 g Karamel zu mir.

Auf den ganzen Tag verteilten sich die Mahlzeiten folgendermaßen:

Morgens zum Frühstück: 30 g Weißbrot mit 30 g Butter, dazu Tee nach Belieben.
Mittags: 100 g mageres gekochtes Rindfleisch, 40 g Weißbrot, Salat aus 200 g grünem Salat und 100 g Tomaten, 20 g Oel und 50 g Zucker..
Abends: 100 g polierter Reis gekocht mit 200 g Milch und 50 g Zucker. Außerdem 30 g Weißbrot mit 30 g Butter. An den Karameltagen nahm ich Karamel auf die einzelnen Mahlzeiten verteilt in Tee zu mir. Außerdem aß ich noch täglich 200 g frische, rohe Aepfel, 2 Zitronen.
Als Getränk nahm ich Tee in beliebigen Mengen zu mir.

Analytischer Teil.

Der Harn wurde gesammelt und seine Menge in 24 Stunden gemessen. In der 24-stündigen Harnmenge wurden bestimmt: der Stickstoff nach der Halbmikromethode von Kjedahl, der Kohlenstoff nach der Mikromethode von Nicloux-Osuka (18), der Vacatsauerstoff nach der Mikromethode von Müller-Kanitz (19).

Versuchstag	Körpergewicht	Harnmenge	Harn C mg.	Harn-Vacat-O mg	Harn-N. mg	C/N	V.O/N.	V.O./C
			Periode 1. Zucker.					
1	85,0	920	7419	11408	7972	0,93	143	1,54
2	85.0	735	8110	12661	9364	0,87	1,34	1,56
3	84.9	750	8285	12893	9461	0,80	1,36	1,56
4	84.9	1400	8434	12020	9996	0,84	1,20	1,42
5	84.9	1080	8729	13549	9540	0,91	1,42	1.55
Periodendurchschnittswert (Dividion der Mittelwerte für C, VO und N ergibt die Mittelwerte d. Quotienten).			8195	12506	9267	0,88	1,35	1,53
			Periode 2. Karamel.					
6	84,9	1200	7187	10543	7845	0,91	1,34	1,47
7	84.9	750	8303	11700	9576	0,87	1,22	1,41
8	84.9	1175	7776	11413	8948	0,87	1,28	1,47
9	84,8	650	5831	11500	7116	0,82	1,62	1,98
10	84,7	985	7446	12676	8274	0,90	1,46	1,61
11	84.7	1060	7333	11235	6752	1,09	1,66	1,53
Periodendurchschnittswert			7309	11396	8085	0.90	1,41	1,56
			Periode 3. Zucker.					
12	84,8	930	6891	10888	7708	0,89	1,41	1,58
13	84,7	600	7031	11160	7694	0,93	1,45	1,59
14	84,7	530	7266	11116	7806	0,93	1.42	1,53
15	84,6	910	8097	12791	9708	0,83	1,32	1,58
Periodendurchschnittswert			7321	11488	8228	0,89	1,40	1,57
			Periode 4. Karamel.					
16	84.6	960	7402	11863	7795	0,95	1,52	1,60
17	84.7	1370	6220	10485	7069	0,88	1,48	1,69
18	84,6	1400	7787	11946	7987	0,97	1,49	1,53
Periodendurchschnittswert			7136	11431	7617	0,94	1,50	1,60

Uebersichtstabelle.

Periode Nr.	Körpergewicht in Kilo	Harn-C mg	Harn Vacat-O mg	Harn-N mg	C/N	V-O/N
I. 100 g Zucker.	85,0	8195	12506	9266	*0,88*	*1,34*
II. Karamel!	84,7	7309	11396	8085	*0,90*	*1,40*
III. Zucker.	84,8	7321	11488	8228	*0,89*	*1,40*
IV. Karamel!	84,6	7136	11431	7617	*0,94*	*1,60*

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Lebenslauf.

Ich, Gustav Adolf Friedrich Bahrs bin am 9. Januar 1911 als Sohn des Lehrers Otto Bahrs zu Hamersleben geboren. Ich besuchte das Reformrealgymnasium in Oschersleben (Bode) und legte Ostern 1930 die Reifeprüfung ab. Darauf studierte ich an der Universität Berlin Medizin, wo ich im Juli 1932 die ärztliche Vorprüfung ablegte. Im Sommersemester 1933 studierte ich in Heidelberg, im Wintersemester 1933/34 in Halle (Saale).) Im Sommer 1934 ging ich wieder nach Berlin zurück, wo ich mein Studium im Januar 1936 mit dem medizinischen Staatsexamen, das ich mit gut bestand, beendete. Während meines praktischen Jahres war ich als Medizinalpraktikant auf der chirurgischen Abteilung des Städt. Krankenhauses Berlin-Pankow, auf der Medizinischen Klinik des Städt. Krankenhauses Magdeburg-Altstadt und an der Städt. Nervenklinik Magdeburg-Sudenburg tätig. Seit meiner Bestallung im Februar 1937 bin ich als Assistenzarzt am Städt. Krankenhaus Haldensleben tätig.

Für die Ueberlassung des Themas sowie für die zahlreichen Anregungen während der Anfertigung meiner Arbeit bin ich Herrn Professor Dr. Bickel dankbarst verpflichtet.

031

SUBACUTE ORAL TOXICITY STUDY IN RATS

ON

CARAMEL COLORINGS 25A-1, 30B-0, and 30F-1

FOR: Union Starch and Refining Company
Granite City, Illinois

Lot Nos. 25A-1, 30B-0, 30F-1

S.A. No. 79105

Peter Chacharonis, Ph.D.

Scientific Associates, Inc.
St. Louis, Missouri

April 5, 1963

SAMPLE DESCRIPTION

Sample Code	25A-1	30B-0	30F-1
Caramel Type	Single Strength Beverage	Single Strength Bakers	Single Strength Bakers
Solids-%	65	67	68
pH	2-3	3-4	2-4
Isoelectric Point, pH	< 2	< 4	< 4
Usage Level in Sodas, grams/liter	3-4	N.A.	N.A.
Carbohydrate Used	Starch Hydroly-zate	Starch Hydroly-zate	Molasses
Catalysts:			
Sulfuric Acid		X	
Ammonium Hydroxide	X	X	X
Sulfurous Acid or SO_2	X		X
Bisulfite Salts			
Sodium Hydroxide	X		
Ammonium Sulfate			
Open or Closed Kettle	Closed	Closed	Open
Nitrogen Used Basis Finished Product, %	1.05	1.4	1.4
Sulfur Used Basis Finished Product, %	1.82	0.1	1.2
Reaction Temp., °F.	280	280	290
Reaction Time, Hrs.	2	1-1/4	1-1/2

INTRODUCTION

The object of this study is to establish adequate safety of Caramel Colorings 25A-1, 30B-0, and 30F-1 after repeated use. The study was designed to determine whether any ill effects and pathological changes are produced in rats after large doses are administered orally over a period of ninety days.

EXPERIMENTAL PROCEDURE:

The Animals. Eighty young Sprague-Dawley albino rats of both sexes were used in this study.

The rats were divided into four groups of 20 rats each and were distributed among the groups by litter, weight, sex, and age.

Maintenance and Diet. All animals were maintained in a uniform environment and housed individually in wire-bottomed cages. The animals were closely observed daily for signs of abnormal behavior and toxic effects. Weight of each animal was recorded weekly, and averages are summarized in Table I.

The Caramel Colorings were administered orally by admixture with the diet at a level of 10% w/w (designed to supply daily caramel coloring dosages of approximately ten grams per kilogram body weight). Ground Purina Laboratory Chow was used in preparing the diets at the above level. Control animals received the same diet without the Caramel Coloring. The appropriate diets and water were supplied ad libitum. Average food consumption is summarized in Table II.

Dosage Levels.

Group I. Caramel Coloring 25A-1
Dosage: 10 grams/Kg body weight/day (10% in diet)

Group II. Caramel Coloring 30B-0
Dosage: 10 grams/Kg body weight/day (10% in diet)

Group III. Caramel Coloring 30F-1
Dosage: 10 grams/Kg body weight/day (10% in diet)

Group IV. Control
(0.0%)

Blood and Urine Studies. Blood counts and urine analyses were made on 50% of the animals (5 males and 5 females from each group) initially, at one, two, and three months. The same animals from each group were used for each determination. The blood tests, using Schilling's classification, included the following:

microhematocrit

hemoglobin

white cell count

differential cell count.

Results are shown in Tables III and IV.

Urine analyses included:

sugar

protein

acetone.

Results are shown in Table V.

Gross Pathology. At the end of the experimental period all the animals were sacrificed by decapitation and examined for gross systemic damage. After gross examination was completed, all the animals were preserved in 10% neutral formalin.

Histopathology. Portions of the following tissues from 5 males and 5 females from each group were sectioned, stained with hematoxylin eosin, and examined microscopically for pathological changes:

small intestine	kidney	stomach (fundic and pyloric)
lung	pancreas	cecum
heart	liver	colon
adrenals	spleen	

Histopathology was performed by W.R. Platt, M.D., F.A.C.P., Pathologist.

RESULTS AND DISCUSSION:

Group I. Caramel Coloring 25A-1

Dosage Level: 10% w/w admixed to diet (approximately 10 grams per kilogram body weight per day)

All animals in this group survived the experimental period and displayed no gross signs of ill effect. Weight gains were within normal limits but slightly lower than those of control animals (Table I.). Food consumption, though greater than that of the Control Group, was within normal limits (Table II.).

The blood picture appears normal and reveals no significant hematological changes as compared to that of the animals of the Control Group (Tables III and IV).

Urine analysis did not reveal significant abnormalities as compared to that of Control animals (Table V).

Autopsy of the sacrificed animals revealed no gross systemic damage.

Microscopic Description

Animal No.1 Sections of liver, kidneys, adrenals, heart, lungs, spleen and gastrointestinal tract from this animal show no significant microscopic changes.

Animal No.3 Sections from the liver, kidneys, adrenals, heart, and lungs are not remarkable except for peribronchiolar lymphocytic infiltration around the lungs. Spleen, gastrointestinal tract show no significant microscopic changes.

Animal No.5 Sections of adrenals show some congestion of the medullary region. The cortex is not remarkable. Sections of the myocardium, liver and kidney are not remarkable. The lungs show interstitial edema and congestion. Spleen, pancreas, and gastrointestinal tract are not remarkable.

Animal No.7 No significant microscopic changes seen in any of the organs examined.

Animal No.9 Sections of the pancreas, adrenals, liver, kidneys, gastrointestinal tract, and spleen and lungs show no significant microscopic changes.

Animal No.11 No microscopic alterations in the sections of liver, kidney, adrenals, heart, lungs, spleen and gastrointestinal tract were present.

Animal No.13 Except for peribronchiolar lymphocytic infiltration, the rest of the organs show no significant microscopic changes.

Animal No.15 The lymphoid-like aggregates seen in previous sections and also the controls seem to be accentuated in the sections of this animal. The rest of the organs show no significant microscopic changes.

Animal No.17 No significant microscopic alterations seen in any of the sections examined.

Animal No.19 Except for interstitial congestion in the kidneys and congestion of the medulla of the adrenals, there are no significant microscopic changes in any of the organs examined.

Group II. Caramel Coloring 30B-0

Dosage Level: 10% w/w admixed to diet (approximately 10 grams per kilogram body weight per day)

All animals in this group survived the experimental period and displayed no gross signs of ill effect. Weight gains were within normal limits but slightly lower than those of Control animals (Table I.). Food consumption, though greater than that of the Control Group was within normal limits (Table II.).

The blood picture appears normal and reveals no significant hematological changes as compared to that of the animals of the Control Group (Tables III. and IV.).

Urine analysis did not reveal significant abnormalities as compared to that of Control animals (Table V.).

Autopsy of the sacrificed animals revealed no gross systemic damage.

Microscopic Description.

Animal No.21 In this animal there appears only to be a minimal congestion interstitially in the lungs and medullary portion of the adrenals; otherwise not remarkable.

Animal No.23 Sections of the lungs show a minimal lymphocytic infiltration with some hyalinization of many of the vessels. The rest of the organs are not remarkable.

Animal No.25 No significant changes seen in the liver, kidneys, adrenals, heart, lungs, spleen, stomach, small intestines, cecum, and colon.

Animal No.27 Sections of the gastrointestinal tract, liver, adrenals, kidney, pancreas, spleen, and lungs, and heart show no significant microscopic changes.

Animal No.29 Sections of the lungs show enlarged lymphoid follicle-like area around the bronchiolar lumen and some interstitial congestion. The rest of the other organs are not remarkable.

Animal No.31 No significant microscopic changes seen in any of the organs examined.

Animal No.33 No significant microscopic changes seen in the adrenals, pancreas, lung, liver, spleen, heart, or gastrointestinal tract.

Animal No.35 No significant microscopic changes seen in any of the tissues examined.

Animal No.37 No significant microscopic changes seen in any of the structures examined.

Animal No.39 Except for lymphocytic peribronchiolar aggregates, the rest of the microscopic structures are not remarkable.

Group III. Caramel Coloring 30F-1

Dosage Level: 10% w/w admixed to diet (approximately 10 grams per kilogram body weight per day)

All animals in this group survived the experimental period and displayed no gross signs of ill effect. Weight gains were within normal limits but slightly lower than those of Control animals (Table I.). Food consumption, though greater than that of the Control Group, was within normal limits (Table II.).

The blood picture appears normal and reveals no significant hematological changes as compared to that of animals of the Control Group (Tables III. and IV.).

Urine analysis did not reveal significant abnormalities as compared to that of Control animals (Table V.).

Autopsy of the sacrificed animals revealed no gross systemic damage.

Microscopic Description.

Animal No.41 Sections of gastrointestinal tract show some submucosal congestion. Sections of the lungs show some interstitial congestion. The interstitial regions of the kidney also congested. Sections of myocardium, cecum and colon, pancreas, spleen, adrenals, and liver show no significant microscopic changes.

Animal No.43 Sections of liver, kidneys, and adrenals show no significant microscopic changes. There is minimal interstitial congestion in the kidneys. Lungs are characterized by focal collections of lymphocytes, mononuclear cells around the bronchi and bronchioles of the sections of lung examined. Spleen shows moderate congestion of the red pulp. Pancreas, heart, stomach, small intestines, cecum and colon are not remarkable.

Animal No.45 Sections of these organs, the liver, kidneys, adrenals, lungs, spleen, pancreas, heart, gastrointestinal tract, are not significant.

Animal No.47 Sections of the spleen, lung, adrenals, kidneys, liver, pancreas, heart, fundic and pyloric region of the stomach, small intestines, and cecum and colon show no significant microscopic changes.

Animal No.49 Sections of adrenals, spleen and lungs, kidney, liver, pancreas, stomach, small intestines, cecum and colon show no significant microscopic changes except for an increase in lymphocytic infiltration around the peribronchiolar regions of the lung tissue.

Animal No.51 No significant microscopic changes seen in any of the sections examined. These include all of the aforementioned structures.

Animal No.53 Except for interstitial congestion in the kidney tissues, no evidence of any specific histopathological changes seen in the liver, kidneys, adrenals, lungs, spleen, pancreas, heart, or gastrointestinal tract.

Animal No.55 No significant histopathological changes seen in any of the organs examined: that is, liver, kidney, lungs, spleen, pancreas, heart, and gastrointestinal tract. The only area in which there appears to be any microscopic changes is lymphocytic infiltration around the peribronchiolar areas.

Animal No.57 Lungs show peribronchiolar lymphocytic infiltration. The kidneys show interstitial congestion. The rest of the organs: gastrointestinal tract, spleen, pancreas, adrenals and liver and heart, are not remarkable microscopically.

Animal No.59 Sections of the liver, adrenals, kidneys, spleen, lungs, pancreas, heart, stomach including fundic and pyloric areas, small intestines, cecum and colon show no significant microscopic changes.

Group IV. Control

Dosage Level: 0%

All animals in this group survived the experimental period and displayed no gross signs of ill effect. Weight gains (Table I.) and food consumption (Table II.) were within normal limits.

The blood picture (Tables III. and IV.) was within normal limits.

Urine analysis (Table V.) did not reveal significant abnormalities with the exception of the occasional appearance of minimal traces of albumin which is considered to be of doubtful significance and within the limits of the experiment.

Autopsy revealed no gross systemic damage.

Microscopic Description.

Animal No.61 Sections of kidneys show interstitial congestion. Sections of liver, adrenals, pancreas, spleen, lungs, heart, gastrointestinal tract, including the stomach, small intestines, cecum and colon, show no significant microscopic changes.

Animal No.63 Sections of lung show focal collections of polys, lymphocytes and mononuclear cells with adjacent areas of interstitial congestion. This is significant of a focal pneumonia which must be a terminal event in this animal. Sections of spleen, pancreas, adrenals, kidney, liver, heart, and gastrointestinal tract are not remarkable.

Animal No.65 Sections of heart, lungs, adrenals, kidneys, pancreas, stomach, liver, gastrointestinal tract show no significant microscopic changes.

Animal No.67 Sections of lungs show definite distinct lymphoid peribronchiolar aggregate as seen in other sections. Sections of the liver are not remarkable. Kidneys show interstitial congestion. Adrenals are not remarkable. Heart, spleen, and gastrointestinal tract are not remarkable.

Animal No.69 Sections of lungs show peribronchiolar lymphocytic infiltration. The rest of the organs show no significant microscopic changes.

Animal No.71 No significant microscopic changes seen in any of the organs examined.

Animal No.73 There is no significant infiltration or degenerative or neoplastic change involving the liver, kidney, adrenals, lungs, spleen, pancreas, heart and gastrointestinal tract of this animal.

Animal No.75 No significant microscopic changes seen in any of the organs examined.

Animal No.77 Sections of the liver are not remarkable. Kidneys show interstitial congestion. Adrenals, lungs, spleen, pancreas, heart, and gastro-intestinal tract are not remarkable.

Animal No.79 Lungs show peribronchiolar lymphocytic infiltration. The rest of the lung tissue is not remarkable. Kidneys show minimal interstitial congestion. Adrenals, liver, spleen, pancreas, heart and stomach, small intestines, cecum and colon are not remarkable.

CONCLUSIONS:

As seen in Table IIa, the cumulative efficiency of food utilization was found to be lower for the experimental groups than for the control. The average gain in grams per gram of food consumed for the various groups is as follows:

	25A-1	30B-0	30F-1	Control
Males	0.143	0.137	0.132	0.169
Females	0.096	0.101	0.094	0.127
Males and Females	0.120	0.119	0.113	0.148
Efficiency as Percent of Control	81.08%	80.41%	76.35%	100%

The above data strongly indicate that the caramel colorings have a questionable nutritive value, if any at all. Moreover, it is of interest to note that, even though weight gains in the experimental groups were less than those in the control, the actual food consumed was greater. A comparison of the equivalent intake of normal basal diet (as shown in Table 1) by each group further illustrates the decrease in food utilization in the experimental groups as compared to controls.

TABLE 1

Comparison of Average Cumulative Efficiency of Food Utilization Based on Equivalent Basal Diet Intake (grams)

	25A-1	30B-0	30F-1	Control
Total Food Consumed	1854.7	1909.5	2003.4	1636.8
Equivalent Normal Basal Rations (Total minus 10% Caramel Coloring)	1669.2	1718.6	1803.1	1636.8
Cumulative Efficiency of Food Utilization (gm Gained per gm Consumed)	0.135	0.134	0.127	0.148
Percent of Control	91.22%	90.54%	85.81%	100%

Evaluation of the overall blood picture (Table IV) reveals no hematological changes resulting from the addition of the Caramel Colorings to the diet at a 10% w/w level. Urine analysis did not reveal significant abnormalities in rats receiving the Caramel Colorings and was comparable to that of the Control animals.

Gross examination of the sacrificed animals revealed no remarkable alterations of vital organs in either experimental or control groups.

Histopathological examination of the tissues did not reveal significant alterations which can be attributed to the ingestion of Caramel Colorings 25A-1, 30B-0 and 30F-1. The occasional and marked lymphocytic peribronchiolar infiltration is seen in control as well as experimental animals, and, as such, is not significant.

SUMMARY:

1. Three groups of Sprague-Dawley albino rats (20 animals per group, 10 males and 10 females) were fed Caramel Colorings 25A-1, 30B-0, and 30F-1 for 90 days at a dietary level of 10% w/w (equivalent to approximately 10 gm/Kg/day). Twenty Sprague-Dawley albino rats, ten of each sex, fed the same diet without Caramel Coloring served as a control group.

2. All animals survived the experimental period and displayed no signs of ill effect.

3. Weight changes of rats in the three experimental groups were slightly less than those of rats in the control group.

4. Cumulative efficiency of food utilization was found to be less for the experimental group as compared to the control.

5. No significant hematological changes were evident in experimental animals as compared to the controls.

6. Urine analysis did not reveal significant abnormalities.

7. Gross and histopathological examination of the sacrificed animals revealed no significant alterations associated with the ingestion of Caramel Colorings 25A-1, 30B-0, and 30F-1 at a 10% w/w dietary level.

SCIENTIFIC ASSOCIATES

TABLE I

Weight Records (grams)

Group I. Caramel Coloring 25A-1

(Initial Date: 10/29/62)

t.	Sex	Init.	Weeks 1	2	3	4	5	6	7	8	9	10	11	12	13	Total Change	% Total Change
1	M	65	120	166	206	232	250	266	290	316	332	338	342	350	350	+285	+438.5
2	M	70	114	158	190	222	265	292	310	336	356	364	370	374	390	+320	+457.1
3	M	75	134	180	204	230	290	306	330	354	374	392	400	394	390	+315	+420.0
4	M	80	126	164	204	218	245	270	288	308	324	334	340	340	350	+270	+337.5
5	M	75	122	160	200	222	245	254	270	296	320	320	340	334	344	+269	+358.7
6	M	85	136	184	234	274	300	328	350	370	390	398	404	376	398	+313	+368.2
7	M	75	110	148	194	226	245	274	298	330	350	360	372	362	388	+313	+417.3
8	M	75	126	162	200	236	250	264	266	300	290	312	326	304	332	+257	+342.7
9	M	95	138	172	186	170	215	270	290	320	336	344	350	342	356	+261	+274.7
0	M	100	146	190	236	270	280	315	330	340	366	376	390	374	392	+292	+292.0
3.	M	79.5	127.2	168.4	205.4	230.0	258.5	283.9	302.2	327.0	343.8	352.8	363.4	355.0	369.0	+289.5	+370.7
1	F	100	140	170	186	198	210	215	224	228	240	236	240	240	246	+146	+146.0
2	F	70	110	136	160	176	180	190	196	206	200	200	210	206	210	+140	+200.0
5	F	70	74	112	142	136	165	170	184	200	208	206	210	208	214	+144	+205.7
4	F	60	98	132	154	162	180	185	206	220	226	230	240	234	240	+180	+300.0
6	F	65	108	138	150	166	180	175	190	210	220	220	226	222	234	+169	+260.0
7	F	75	112	140	166	174	185	185	210	202	212	220	226	216	228	+153	+204.0
8	F	70	116	150	174	186	200	210	230	238	244	242	250	242	246	+176	+251.4
9	F	65	108	134	136	150	165	174	196	210	214	222	228	224	234	+169	+260.0
0	F	70	84	132	154	174	180	180	204	216	218	216	220	220	220	+150	+214.3
5.	F	55	106	130	156	146	180	180	202	210	222	226	232	226	234	+179	+325.5
	F	70.0	105.6	137.4	158.6	166.8	182.5	186.4	204.2	214.0	220.4	221.8	228.2	223.8	230.6	+160.6	+236.7
.M&F		74.8	116.4	152.9	182.0	198.4	220.5	235.2	253.2	270.5	282.1	287.3	295.8	289.4	299.8	+225.1	+303.7

TABLE I

Weight Records (grams) - continued

Group II. Caramel Coloring 30B-0

t.	Sex	Init.	Weeks 1	2	3	4	5	6	7	8	9	10	11	12	13	Total Change	% Total Change
1	M	85	130	180	222	244	270	274	300	328	364	364	370	378	378	+293	+344.7
2	M	80	136	182	228	240	260	272	310	336	354	358	364	358	376	+296	+370.0
3	M	75	110	140	166	180	190	194	208	230	240	252	266	268	280	+205	+273.3
4	M	80	118	162	214	200	240	286	310	334	370	376	386	386	400	+320	+400.0
5	M	90	132	176	218	222	265	284	316	340	372	372	384	382	390	+300	+333.3
6	M	85	126	160	192	214	240	250	266	286	294	312	320	316	322	+237	+278.8
7	M	75	116	163	204	226	245	264	296	333	374	390	400	400	406	+331	+441.3
8	M	90	138	184	232	224	270	290	314	332	346	356	368	360	376	+286	+317.8
9	M	75	124	160	200	230	260	274	296	326	350	368	384	384	390	+315	+420.0
0	M	110	166	200	236	228	290	302	320	342	366	372	374	354	382	+272	+247.3
.	M	84.5	129.6	170.6	211.2	220.8	253.0	269.0	293.6	319.2	343.0	352.0	351.6	358.6	370.0	+285.5	+342.7
1	F	65	112	140	158	176	170	190	202	220	232	232	238	230	236	+171	+263.1
2	F	60	110	138	160	170	185	180	192	193	210	206	218	212	218	+158	+263.3
5	F	60	100	136	170	150	190	196	212	220	240	240	240	238	236	+176	+293.3
4	F	65	110	138	160	150	175	184	200	204	216	220	224	218	220	+155	+238.5
5	F	60	110	136	126	170	185	184	196	212	222	224	236	238	244	+184	+306.7
6	F	80	120	150	170	194	200	212	218	230	240	240	252	260	262	+182	+227.5
7	F	95	118	132	160	182	200	208	230	248	260	258	266	268	264	+169	+177.9
8	F	55	98	120	150	158	170	170	178	188	194	198	206	202	208	+153	+278.2
9	F	95	140	170	188	218	240	246	266	280	296	290	300	296	300	+205	+215.8
0	F	60	106	138	156	172	185	192	210	220	234	236	240	234	244	+184	+306.7
.	F	69.5	112.4	139.8	159.8	174.0	190.0	196.2	210.4	222.0	234.4	234.4	242.0	239.6	243.2	+173.7	+257.1
	M+F	77.0	121.0	155.2	185.5	197.4	221.5	232.6	252.0	270.6	288.7	293.2	296.8	299.1	306.6	+229.6	+299.9

TABLE I

Weight Records (grams) - continued

Group III. Caramel Coloring 30F-1

Rat No.	Sex	Init.	Weeks 1	2	3	4	5	6	7	8	9	10	11	12	13	Total Change	% Total Change
41	M	80	124	150	166	176	215	240	262	278	298	300	314	304	324	+244	+305.0
42	M	80	136	168	186	204	260	286	314	344	366	374	386	356	388	+308	+385.0
43	M	70	126	142	158	184	250	264	290	318	338	336	342	332	354	+284	+405.7
44	M	90	136	174	180	196	240	246	268	284	294	296	316	306	338	+248	+275.6
45	M	80	136	152	176	170	200	260	292	324	346	356	376	370	386	+306	+382.5
46	M	70	110	146	174	204	230	250	276	302	328	346	358	354	370	+300	+428.6
47	M	85	130	166	172	200	270	284	322	354	380	274	386	366	392	+309	+363.5
48	M	80	130	164	180	212	235	260	274	296	312	330	346	314	350	+270	+337.5
49	M	85	132	170	170	200	265	290	310	340	358	360	380	358	388	+303	+356.5
50	M	105	150	180	190	204	270	284	310	332	352	364	384	374	390	+285	+271.4
Avg	M	82.5	131.0	161.2	175.4	195.0	243.5	266.4	291.8	317.2	337.2	343.6	358.8	343.4	368.2	+285.7	+351.1
51	F	75	116	120	114	144	190	196	212	224	236	236	238	234	242	+167	+222.7
52	F	85	120	144	160	178	186	186	206	212	220	230	234	236	234	+149	+175.3
53	F	70	114	152	152	190	230	240	274	294	316	320	334	326	348	+278	+397.1
54	F	60	96	126	152	154	134	170	182	196	202	204	214	206	214	+154	+256.7
55	F	55	88	124	142	160	170	176	192	200	210	210	218	218	222	+176	+303.6
56	F	60	102	130	150	166	170	196	202	208	216	222	234	224	232	+172	+286.7
57	F	95	146	178	136	172	205	224	248	250	260	260	266	254	260	+165	+173.7
58	F	70	100	124	146	166	175	188	204	212	220	226	230	218	232	+162	+231.4
59	F	65	112	142	142	150	190	200	214	216	232	232	240	232	242	+177	+272.3
60	F	105	152	170	166	178	185	216	230	246	252	252	252	256	252	+147	+140.0
Avg	F	74.0	114.6	141.0	146.0	165.8	183.5	199.2	216.4	225.8	236.4	239.2	246.0	240.4	247.8	+173.8	+246.0
Avg	M+F	78.3	122.8	151.1	160.7	180.4	213.5	232.8	254.1	271.5	286.8	291.4	302.4	291.9	308.0	+229.8	+298.6

TABLE I

Weight Records (grams) - continued

Group IV. Control

Rat No.	Sex	Init.	Weeks 1	2	3	4	5	6	7	8	9	10	11	12	13	Total Change	% Total Change
61	M	80	128	168	210	246	270	285	325	354	374	380	396	386	395	+315	+393.7
62	M	75	122	168	202	222	245	260	288	310	332	344	352	352	362	+287	+382.6
63	M	75	112	156	202	242	260	275	300	330	344	350	366	360	378	+303	+404.0
64	M	80	120	160	206	230	265	280	302	324	346	356	364	364	382	+302	+377.5
65	M	85	136	176	232	276	310	330	360	396	414	430	446	436	460	+375	+441.2
66	M	80	134	178	226	260	280	300	332	356	378	390	392	396	406	+326	+407.5
67	M	70	120	162	204	228	255	270	298	324	354	368	386	386	398	+328	+468.6
68	M	75	128	172	226	256	270	290	310	332	346	356	370	372	384	+309	+412.0
69	M	75	110	148	194	216	240	250	280	310	330	334	348	352	356	+281	+374.7
70	M	110	160	196	238	260	275	290	304	314	332	336	340	344	346	+236	+214.6
Avg	M	80.5	127.0	168.4	214.0	243.6	267.0	283.0	309.9	335.0	355.0	364.4	376.0	374.8	386.7	+306.2	+387.6
71	F	80	126	156	184	204	210	220	230	242	256	258	266	264	268	+188	235.0
72	F	70	116	150	172	186	200	205	220	232	240	242	242	246	250	+180	+257.1
73	F	105	134	156	174	186	195	190	206	220	224	224	230	232	230	+125	+119.1
74	F	70	126	156	176	196	205	205	234	250	258	258	266	254	266	+196	+280.0
75	F	70	114	144	160	180	195	200	214	230	240	242	250	246	256	+186	+265.7
76	F	65	106	136	160	172	190	196	216	232	240	244	250	250	264	+199	+306.2
77	F	65	160	190	212	220	225	226	238	254	260	264	270	272	280	+215	+330.8
78	F	75	120	140	164	180	195	200	210	226	240	240	242	242	246	+171	+228.0
79	F	65	110	140	168	194	205	212	230	240	250	250	254	258	266	+201	+309.2
80	F	55	108	140	166	184	190	204	216	226	232	242	246	246	260	+205	+372.7
Avg	F	72.0	122.0	150.8	173.6	190.2	201.0	205.8	221.4	235.2	244.8	246.4	251.6	251.0	258.6	+186.6	+270.4
Avg	M+F	76.3	124.5	159.6	193.8	216.9	234.0	244.4	265.7	285.1	299.9	305.4	313.8	312.9	322.7	+246.4	+329.0

TABLE II

Food Consumption (grams)

Group I. Caramel Coloring 25A-1 - 10% w/w by admixture to diet - -

Rat No.	Sex	Weeks 1	2	3	4	5	6	7	8	9	10	11	12	13	Total Food Consumption	Average Weekly Food Consumption
1	M	119	135	145	140	160	185	175	170	175	150	140	150	130	1974	151.84
2	M	119	145	140	145	190	200	190	185	190	165	160	170	160	2159	166.07
3	M	131	150	155	160	155	155	200	180	190	170	180	170	150	2146	165.07
4	M	115	125	145	160	170	180	185	180	160	150	150	150	140	2010	154.61
5	M	125	145	145	135	165	185	185	170	170	150	155	140	140	2010	154.61
6	M	121	150	150	150	185	170	180	175	180	170	154	140	150	2036	160.46
7	M	89	115	130	135	155	140	180	160	170	155	160	140	130	1859	143.00
8	M	125	125	125	135	145	185	145	145	155	170	130	125	125	1835	141.15
9	M	135	135	155	155	160	165	195	180	185	160	160	130	135	2050	157.69
10	M	145	145	135	150	160	175	200	165	170	160	160	140	155	2060	158.46
Avg	M	122.4	137.0	142.5	146.5	164.5	174.0	183.5	171.0	174.5	160.0	156.0	145.5	141.5	2018.9	155.30
11	F	119	130	135	115	125	160	155	145	140	125	130	130	115	1724	132.61
12	F	111	115	135	115	125	145	135	115	105	105	120	110	115	1551	119.30
13	F	91	115	115	120	130	155	140	185	165	160	160	160	145	1841	141.61
14	F	101	135	120	95	130	130	135	140	145	135	125	110	100	1601	123.15
15	F	105	135	115	100	165	155	140	140	120	100	105	100	90	1570	120.76
16	F	115	130	115	105	135	130	140	110	110	115	110	100	105	1520	116.92
17	F	119	140	135	120	130	155	150	140	140	125	125	105	120	1704	131.07
18	F	100	125	95	95	115	160	175	210	210	170	125	120	115	1815	139.61
19	F	155	115	110	115	130	165	175	165	145	135	165	125	135	1835	141.15
20	F	115	135	150	100	145	150	170	140	135	130	140	115	120	1745	134.23
Avg	F	113.1	127.5	122.5	108.0	133.0	150.5	151.5	149.0	141.5	130.0	130.5	117.5	116.0	1690.6	130.04
Avg	M+F	117.75	132.25	132.50	127.25	147.75	162.25	167.50	160.00	158.00	145.00	143.30	131.50	128.75	1854.75	142.66

TABLE II

Food Consumption (grams) - continued

Group II. Caramel Coloring 30B-0 - 10% w/w by admixture to diet

Rat No.	Sex	Weeks 1	2	3	4	5	6	7	8	9	10	11	12	13	Total Food Consumption	Average Weekly Food Consumption
21	M	105	130	135	160	175	165	160	180	200	165	160	160	150	2045	157.30
22	M	125	135	145	160	160	115	195	190	200	170	165	160	160	2080	160.00
23	M	100	125	125	140	115	160	140	180	160	140	135	140	160	1820	140.00
24	M	119	135	145	160	180	200	195	230	230	220	210	180	190	2394	184.15
25	M	119	110	135	165	185	160	175	190	220	170	170	160	160	2119	163.00
26	M	115	115	110	155	155	140	160	155	160	150	140	140	150	1845	141.92
27	M	113	120	130	150	145	195	210	210	220	190	180	175	190	2228	171.38
28	M	123	140	155	170	185	130	205	200	200	180	170	150	150	2158	166.00
29	M	103	115	115	150	150	160	160	170	170	170	165	165	165	1958	150.61
30	M	135	155	155	160	205	190	200	180	175	160	150	140	150	2155	165.76
Avg	M	115.7	128.0	135.0	157.0	165.5	161.5	180.0	188.5	193.5	171.5	164.5	157.0	162.5	2080.2	160.01
31	F	119	100	95	125	120	155	155	150	140	140	180	130	130	1739	133.76
32	F	115	110	85	115	105	145	120	130	130	105	105	110	110	1485	114.23
33	F	150	125	115	160	205	160	170	180	190	200	185	180	180	2200	169.23
34	F	105	110	110	150	135	145	140	130	160	130	110	90	100	1615	124.23
35	F	105	110	155	150	205	130	130	130	130	135	130	135	120	1765	135.76
36	F	105	115	100	120	120	130	140	140	130	130	130	120	125	1605	123.46
37	F	85	95	110	135	125	170	170	170	155	140	140	120	125	1740	133.84
38	F	91	115	115	135	135	155	155	155	200	155	170	135	145	1861	143.15
39	F	120	135	125	135	145	145	150	180	165	140	140	130	120	1830	140.76
40	F	89	105	90	120	135	85	130	130	140	140	130	120	135	1549	119.15
Avg	F	108.4	112.0	110.0	134.5	143.0	142.0	146.0	149.5	154.0	141.5	142.0	127.0	129.0	1738.9	133.76
Avg	M+F	112.05	120.0	122.50	145.75	154.25	151.75	163.00	169.00	173.75	156.50	153.25	142.00	147.10	1909.55	146.88

TABLE II

Food Consumption (grams) - continued

Group III. Caramel Coloring 30F-1 - 10% w/w by admixture to diet

Rat No.	Sex	Weeks 1	2	3	4	5	6	7	8	9	10	11	12	13	Total Food Consumption	Average Weekly Food Consumption
41	M	105	120	150	155	155	160	160	140	155	140	140	130	140	1850	142.30
42	M	130	155	160	170	200	200	185	180	190	175	170	130	140	2205	169.61
43	M	135	155	155	175	200	160	200	190	185	155	150	140	150	2150	165.38
44	M	111	145	165	165	190	175	170	180	165	160	155	130	155	2066	158.92
45	M	105	155	160	175	180	190	195	180	200	180	180	150	170	2220	170.76
46	M	95	140	160	180	180	225	165	170	175	170	150	130	140	2080	160.00
47	M	113	155	180	200	205	205	220	210	230	190	190	150	160	2408	185.23
48	M	125	155	165	155	145	175	155	140	180	170	155	125	150	1995	153.46
49	M	111	145	175	200	240	185	180	180	220	180	170	150	155	2291	176.23
50	M	135	155	170	190	220	220	180	180	200	180	170	160	170	2330	179.23
Avg	M	116.5	148.0	164.0	176.5	191.5	189.5	181.0	175.0	190.0	170.0	163.0	139.5	155.0	2159.5	166.11
51	F	100	155	155	160	180	175	125	140	165	120	130	110	120	1835	141.15
52	F	89	125	150	145	135	140	130	130	130	125	125	110	90	1624	124.92
53	F	111	150	155	170	185	175	175	170	190	180	170	165	160	2156	165.84
54	F	91	115	145	140	155	165	140	140	140	130	120	110	110	1701	130.84
55	F	155	125	140	150	160	165	120	180	200	180	150	100	90	1915	147.80
56	F	95	135	150	140	155	160	140	130	170	150	155	130	155	1865	143.46
57	F	111	155	155	155	160	170	160	150	150	140	130	100	110	1846	142.00
58	F	85	110	135	130	115	160	135	130	130	125	110	105	100	1570	120.76
59	F	105	145	155	170	195	180	150	180	200	160	165	130	120	2055	158.07
60	F	127	150	155	160	160	165	160	160	160	155	130	115	110	1907	146.69
Avg	F	106.9	136.5	149.5	152.0	160.0	165.5	143.5	151.0	163.5	146.5	138.5	117.5	116.5	1847.4	142.10
Avg	M+F	111.70	142.25	156.75	164.25	175.75	177.50	162.25	163.00	176.75	158.25	150.75	128.50	135.75	2003.45	154.10

TABLE II

Food Consumption (grams) - continued

Group IV. Control

Rat No.	Sex	Weeks 1	2	3	4	5	6	7	8	9	10	11	12	13	Total Food Consumption	Average Weekly Fo Consumpti
61	M	111	105	135	160	155	150	170	175	170	140	165	140	160	1936	148.92
62	M	95	115	110	140	125	130	155	150	150	145	135	130	130	1710	132.53
63	M	85	100	125	160	130	130	155	155	150	140	135	130	120	1715	131.92
64	M	110	105	125	130	125	130	150	150	150	150	145	130	125	1725	132.69
65	M	91	115	140	180	155	150	180	180	180	170	160	160	155	2016	155.07
66	M	115	95	125	160	145	160	160	170	170	165	150	140	140	1895	145.76
67	M	109	130	145	160	135	125	160	160	150	150	150	135	130	1839	141.46
68	M	99	110	130	170	145	145	150	160	155	150	150	140	140	1844	141.84
69	M	119	85	115	135	125	125	155	150	150	140	130	125	125	1679	129.15
70	M	95	105	120	155	215	140	150	140	140	130	120	110	115	1735	133.46
Avg	M	102.9	106.5	127.0	155.0	145.5	138.5	158.5	159.0	156.5	148.0	144.0	134.0	134.0	1809.4	139.18
71	F	95	115	105	140	120	120	170	160	165	115	120	100	120	164.5	126.53
72	F	89	100	115	120	105	85	120	115	115	120	115	105	115	1419	109.15
73	F	85	75	80	120	95	85	110	115	105	100	90	100	100	1260	96.92
74	F	95	110	105	140	125	130	160	140	130	125	130	100	120	1610	123.84
75	F	85	90	85	125	105	105	125	130	130	110	110	105	105	1410	108.46
76	F	69	80	85	130	105	105	140	120	120	110	105	110	120	1399	107.61
77	F	131	150	125	120	105	100	160	135	135	110	115	120	125	1631	125.46
78	F	95	75	80	115	105	95	145	115	120	110	110	100	105	1370	105.38
79	F	99	80	95	130	145	95	140	130	125	100	110	105	120	1474	113.38
80	F	95	100	115	120	105	105	120	120	120	105	100	100	120	1425	109.61
Avg	F	93.8	97.5	99.0	126.0	111.5	102.5	139.0	128.0	126.5	110.5	110.5	104.5	115.0	1464.3	112.63
Avg	M+F	98.35	102.00	113.00	140.50	128.50	120.50	148.75	143.50	141.50	129.25	127.25	119.25	124.50	1636.85	125.90

TABLE IIa

Food Utilization

Group I. Caramel Coloring 25A-1

Rat No.	Sex	Total Weight Gain (grams)	Total Food Consumed (grams)	Cumulative Conversion: grams of Food Consumed per Gram Body Weight Gain	Cumulative Efficiency of Food Utilization: Grams Gained per Gram of Food Consumed
1	M	281	1974	7.02	0.142
2	M	320	2159	6.75	0.148
3	M	315	2146	6.81	0.147
4	M	270	2010	7.44	0.134
5	M	269	2010	7.47	0.134
6	M	313	2086	6.66	0.150
7	M	313	1859	5.94	0.168
8	M	257	1835	7.14	0.140
9	M	261	2050	7.85	0.127
10	M	292	2060	7.05	0.142
Average	M	289.5	2018.9	7.01	0.143
11	F	146	1724	11.81	0.085
12	F	140	1551	11.08	0.090
13	F	144	1841	12.78	0.078
14	F	180	1601	8.89	0.112
15	F	169	1570	9.29	0.108
16	F	153	1520	9.93	0.101
17	F	176	1704	9.68	0.103
18	F	169	1815	10.74	0.093
19	F	150	1835	12.23	0.082
20	F	179	1745	9.75	0.103
Average	F	160.6	1690.6	10.62	0.096
Average	M+F	225.1	1854.7	8.82	0.120

TABLE IIa

Food Utilization (continued)

Group II. Caramel Coloring 30B-0

Rat No.	Sex	Total Weight Gain (grams)	Total Food Consumed (grams)	Cumulative Conversion: Grams of Food Consumed per Gram Body Weight Gain	Cumulative Effciency of Food Utilization: Grams Gained per Gram of Food Consumed
21	M	293	2045	6.98	0.143
22	M	296	2080	7.03	0.142
23	M	205	1820	8.88	0.113
24	M	320	2394	7.48	0.134
25	M	300	2119	7.06	0.142
26	M	237	1845	7.78	0.128
27	M	331	2228	6.73	0.149
28	M	286	2158	7.55	0.133
29	M	315	1958	6.22	0.161
30	M	272	2155	7.92	0.126
Average	M	285.5	2080.2	7.36	0.137
31	F	171	1739	10.17	0.098
32	F	158	1485	9.40	0.106
33	F	176	2200	12.50	0.080
34	F	155	1615	10.42	0.096
35	F	184	1765	9.59	0.104
36	F	182	1605	8.82	0.113
37	F	169	1740	10.30	0.097
38	F	153	1861	12.16	0.082
39	F	205	1830	8.93	0.112
40	F	184	1549	8.42	0.119
Average	F	173.7	1738.9	10.07	0.101
Average	M+F	229.6	1909.5	8.72	0.119

TABLE IIa

Food Utilization (continued)

Group III. Caramel Coloring 30F-1

Rat No.	Sex	Total Weight Gain (grams)	Total Food Consumed (grams)	Cumulative Conversion: Grams of Food Consumed per Gram Body Weight Gain	Cumulative Efficiency of Food Utilization: Grams Gained per Gram of Food Consumed
41	M	244	1850	7.58	0.132
42	M	308	2205	7.16	0.140
43	M	284	2150	7.57	0.132
44	M	248	2066	8.33	0.120
45	M	306	2220	7.25	0.138
46	M	300	2080	6.93	0.144
47	M	309	2408	7.79	0.128
48	M	270	1995	7.39	0.135
49	M	303	2291	7.56	0.132
50	M	285	2330	8.18	0.122
Average	M	285.7	2159.5	7.57	0.132
51	F	167	1835	10.99	0.091
52	F	149	1624	10.90	0.092
53	F	278	2156	7.76	0.129
54	F	154	1701	11.05	0.091
55	F	167	1915	11.47	0.087
56	F	172	1865	10.84	0.092
57	F	165	1846	11.19	0.089
58	F	162	1570	9.69	0.103
59	F	177	2077	11.73	0.085
60	F	147	1907	12.97	0.077
Average	F	173.8	1847.4	10.86	0.094
Average	M+F	229.8	2003.4	9.22	0.113

TABLE IIa

Food Utilization (continued)

Group IV. Control

Rat No.	Sex	Total Weight Gain (grams)	Total Food Consumed (grams)	Cumulative Conversion: Grams of Food Consumed per Gram Body Weight Gain	Cumulative Efficiency of Food Utilization: Grams Gained per Gram of Food Consumed
61	M	315	1936	6.15	0.163
62	M	287	1710	5.96	0.169
63	M	303	1715	5.66	0.177
64	M	302	1725	5.71	0.175
65	M	375	2016	5.38	0.186
66	M	326	1895	5.81	0.172
67	M	328	1839	5.61	0.178
68	M	309	1844	5.97	0.168
69	M	281	1679	5.98	0.167
70	M	236	1735	7.35	0.136
Average	M	306.2	1809.4	5.96	0.169
71	F	188	1645	8.75	0.114
72	F	180	1419	7.88	0.127
73	F	125	1260	10.08	0.099
74	F	196	1610	8.21	0.122
75	F	186	1410	7.58	0.132
76	F	199	1399	7.03	0.142
77	F	215	1631	7.59	0.132
78	F	171	1370	8.01	0.125
79	F	201	1474	7.33	0.136
80	F	205	1425	6.95	0.144
Average	F	186.6	1464.3	7.94	0.127
Average	M+F	246.4	1636.8	6.95	0.148

TABLE III

Blood Analysis

Group I. Caramel Coloring 25A-1 - - MALES

Rat No.	Time	Hemoglobin grams %	Hematocrit vol. %	WBC	Differential Count							
					Eosinophiles	Basophiles	Myelocytes	Juveniles	Stab	Segmented	Lymphocytes	Monocytes
1	Initial	15.5	45	11,250	0	0	0	0	0	5	94	1
	1 month	16.0	51	20,900	5	0	0	0	0	12	82	1
	2 months	15.5	48	15,500	5	0	0	0	0	8	87	0
	3 months	18.0	54	11,700	1	0	0	0	0	10	88	1
3	Initial	13.8	45	8,650	0	0	0	0	0	6	94	0
	1 month	14.3	46	15,600	5	0	0	0	0	16	79	0
	2 months	14.5	50	18,000	4	0	0	0	0	14	82	0
	3 months	15.5	50	12,300	2	0	0	0	0	8	90	0
5	Initial	13.0	47	17,550	0	0	0	0	0	11	88	1
	1 month	14.7	50	18,650	1	0	0	0	0	16	83	0
	2 months	15.0	50	18,450	6	0	0	0	0	7	87	0
	3 months	15.5	51	20,650	4	0	0	0	0	10	86	0
7	Initial	15.5	53	16,200	0	0	0	0	0	11	87	2
	1 month	14.7	50	14,450	3	0	0	0	0	16	80	1
	2 months	15.2	51	12,400	1	0	0	0	0	12	87	0
	3 months	16.5	52	14,250	3	0	0	0	0	12	85	0
9	Initial	12.5	41	14,200	0	0	0	0	0	10	90	0
	1 month	16.0	51	17,400	3	0	0	0	0	10	87	0
	2 months	14.0	48	12,150	2	0	0	0	0	19	79	0
	3 months	14.5	46	13,750	2	0	0	0	0	14	84	0
Average	Initial	14.06	44.20	13,570	0.0	0.0	0.0	0.0	0.0	8.6	90.6	0.8
	1 month	15.14	49.60	17,400	3.4	0.0	0.0	0.0	0.0	14.0	82.2	0.4
	2 months	14.84	49.40	15,300	3.6	0.0	0.0	0.0	0.0	12.0	84.4	0.0
	3 months	16.00	50.60	14,350	2.4	0.0	0.0	0.0	0.0	10.8	86.6	0.2

TABLE III

Blood Analysis (continued)

Group I. Caramel Coloring 25A-1 - - FEMALES

Rat no.	Time	Hemoglobin grams %	Hematocrit vol. %	WBC	Differential Count							
					Eosinophiles	Basophiles	Myelocytes	Juveniles	Stab	Segmented	Lymphocytes	Monocytes
11	Initial	14.3	48	8,700	1	0	0	0	0	11	88	0
	1 month	15.0	45	11,150	2	0	0	0	0	15	83	0
	2 months	16.5	48	7,600	7	0	0	0	0	8	85	0
	3 months	15.0	49	9,450	2	0	0	0	0	6	92	0
13	Initial	15.0	52	12,750	0	0	0	0	0	15	85	0
	1 month	14.3	44	15,850	3	0	0	0	0	7	90	0
	2 months	15.7	52	22,950	2	0	0	0	0	12	86	0
	3 months	15.0	48	17,550	0	0	0	0	0	8	92	0
15	Initial	15.0	53	10,650	0	0	0	0	0	7	92	1
	1 month	15.5	50	19,950	4	0	0	0	0	7	89	0
	2 months	15.5	47	13,250	2	0	0	0	0	8	90	0
	3 months	15.2	50	13,700	4	0	0	0	0	5	91	0
17	Initial	13.0	40	15,350	0	0	0	0	0	11	89	0
	1 month	14.0	47	17,900	6	0	0	0	0	7	87	0
	2 months	14.0	46	11,150	2	0	0	0	0	12	86	0
	3 months	15.0	46	13,350	2	0	0	0	0	5	92	1
19	Initial	13.0	44	17,550	1	0	0	0	0	5	93	1
	1 month	15.7	48	19,100	2	0	0	0	0	12	85	1
	2 months	15.0	50	16,850	1	0	0	0	0	10	88	1
	3 months	15.7	51	13,300	2	0	0	0	0	12	86	0
Average	Initial	14.06	47.40	13,000	0.4	0.0	0.0	0.0	0.0	9.8	89.4	0.4
	1 month	14.90	46.80	16,790	3.4	0.0	0.0	0.0	0.0	9.6	86.8	0.2
	2 months	15.34	48.60	14,360	2.8	0.0	0.0	0.0	0.0	10.0	87.0	0.2
	3 months	15.80	48.80	13,460	2.0	0.0	0.0	0.0	0.0	7.2	90.6	0.2

TABLE III

Blood Analysis (continued)

Group II. Caramel Coloring 30B-0 - - MALES

Rat No.	Time	Hemoglobin grams %	Hematocrit vol. %	WBC	Differential Count							
					Eosinophiles	Basophiles	Myelocytes	Juveniles	Stab	Segmented	Lymphocytes	Monocytes
21	Initial	13.0	42	26,050	1	0	0	0	0	40	59	0
	1 month	15.0	50	19,900	0	0	0	0	0	17	83	0
	2 months	14.5	48	14,150	3	0	0	0	0	29	68	0
	3 months	15.5	51	15,400	8	0	0	0	0	22	69	1
23	Initial	14.0	51	14,650	0	0	0	0	0	8	91	1
	1 month	15.5	51	15,700	3	0	0	0	0	18	79	0
	2 months	14.5	51	11,800	5	0	0	0	0	21	74	0
	3 months	16.5	53	13,000	1	0	0	0	0	26	73	0
25	Initial	13.2	46	21,000	0	0	0	0	0	9	90	1
	1 month	14.7	51	13,400	2	0	0	0	0	25	73	0
	2 months	14.7	51	18,100	3	0	0	0	0	15	82	0
	3 months	15.5	52	24,900	0	0	0	0	0	6	94	0
27	Initial	13.5	47	14,400	0	0	0	0	0	9	91	0
	1 month	14.5	51	12,750	1	0	0	0	0	13	85	1
	2 months	15.0	50	16,600	4	0	0	0	1	25	70	0
	3 months	16.0	54	16,050	4	0	0	0	0	29	67	0
29	Initial	13.0	44	20,100	0	0	0	0	0	11	89	0
	1 month	15.0	48	18,350	6	0	0	0	0	28	66	0
	2 months	14.7	43	18,900	6	0	0	0	0	15	79	0
	3 months	15.0	50	16,450	6	0	0	0	0	12	82	0
Average	Initial	13.34	46.00	19,240	0.2	0.0	0.0	0.0	0.0	15.4	84.0	0.4
	1 month	14.94	50.20	16,020	2.4	0.0	0.0	0.0	0.0	20.2	77.2	0.2
	2 months	14.68	48.60	15,910	4.2	0.0	0.0	0.0	0.2	21.0	74.6	0.0
	3 months	15.70	52.00	17,160	3.8	0.0	0.0	0.0	0.0	19.0	77.0	0.2

TABLE III

Blood Analysis (continued)

Group II. Caramel Coloring 30B-0 - - FEMALES

Rat No.	Time	Hemoglobin grams %	Hematocrit vol. %	WBC	Differential Count							
					Eosinophiles	Basophiles	Myelocytes	Juveniles	Stab	Segmented	Lymphocytes	Monocytes
31	Initial	14.5	49	12,550	1	0	0	0	0	9	90	0
	1 month	15.1	50	14,300	6	0	0	0	0	12	82	0
	2 months	16.0	50	14,750	2	0	0	0	0	18	80	0
	3 months	16.0	52	15,200	5	0	0	0	0	22	73	0
33	Initial	15.0	47	14,600	0	0	0	0	0	14	85	1
	1 month	14.5	47	11,750	1	0	0	0	0	25	74	0
	2 months	14.0	46	7,150	0	0	0	0	0	12	88	0
	3 months	15.8	49	8,450	1	0	0	0	0	30	69	0
35	Initial	13.5	47	15,300	2	0	0	0	0	19	79	0
	1 month	16.0	50	15,450	4	0	0	0	0	20	76	0
	2 months	14.5	46	14,150	2	0	0	0	0	19	79	0
	3 months	15.5	49	10,900	8	0	0	0	0	15	77	0
37	Initial	14.5	50	16,150	1	0	0	0	0	5	94	0
	1 month	12.5	41	13,450	0	0	0	0	0	11	89	0
	2 months	15.0	50	15,850	1	0	0	0	0	15	83	1
	3 months	14.5	46	14,100	4	0	0	0	0	21	75	0
39	Initial	15.0	48	12,250	0	0	0	0	0	7	93	0
	1 month	15.5	46	13,150	4	0	0	0	0	8	88	0
	2 months	15.5	48	14,350	1	0	0	0	0	11	88	0
	3 months	15.3	48	9,650	1	0	0	0	0	22	77	0
Average	Initial	14.50	48.20	14,170	0.8	0.0	0.0	0.0	0.0	10.8	88.2	0.2
	1 month	14.72	46.80	13,620	3.0	0.0	0.0	0.0	0.0	15.2	81.8	0.0
	2 months	15.00	48.00	13,250	1.2	0.0	0.0	0.0	0.0	15.0	83.6	0.2
	3 months	15.82	48.80	11,660	3.8	0.0	0.0	0.0	0.0	22.0	74.2	0.0

TABLE III

Blood Analysis (continued)

[illegible] Coloring 30F-1 - - MALES

		Hemo-globin grams %	Hemato-crit vol. %	WBC	Differential Count							
					Eosino-philes	Baso-philes	Myelo-cytes	Juve-niles	Stab	Seg-mented	Lympho-cytes	Mono-cytes
	[illegible]	[illegible]	47	17,900	0	0	0	0	0	8	92	0
	[illegible]	13.[illegible]	46	16,100	3	0	0	0	0	21	76	0
	[illegible]	[illegible]	[illegible]	12,250	3	0	0	0	0	20	77	0
	[illegible]	14.2	51	17,800	1	0	0	0	0	11	88	0
35	[illegible]	13.5	45	19,500	0	0	0	0	0	11	89	0
	1 month	14.5	46	15,350	1	0	0	0	0	15	84	0
	2 months	15.2	46	8,800	1	0	0	0	0	22	77	0
	3 months	14.5	45	11,150	0	0	0	0	0	13	86	1
45	Initial	15.3	51	13,250	1	0	0	0	0	15	83	1
	1 month	14.8	47	12,550	1	0	0	0	0	21	77	1
	2 months	15.[illegible]	46	8,850	3	0	0	0	0	12	85	0
	3 months	14.2	48	11,900	5	0	0	0	0	14	83	0
47	Initial	13.[illegible]	45	18,000	0	0	[illegible]	0	0	20	80	0
	1 month	15.2	51	21,250	2	0	0	0	[illegible]	13	85	0
	2 months	15.[illegible]	51	18,650	3	0	0	0	0	11	85	1
	3 months	16.2	53	15,950	3	0	0	0	0	8	89	0
49	Initial	14.5	48	12,350	1	0	0	0	0	8	91	0
	1 month	14.5	45	20,000	3	0	0	0	0	11	86	0
	2 months	15.2	48	22,700	1	0	0	0	0	7	92	0
	3 months	14.5	46	17,650	3	0	0	0	0	5	92	0
[illegible]	Initial	13.86	47.20	16,200	0.4	0.0	0.0	0.0	0.0	12.4	87.0	0.2
	1 month	14.50	47.00	17,450	2.0	0.0	0.0	0.0	0.0	16.2	81.6	0.2
	2 months	15.48	48.60	14,250	2.2	0.0	0.0	0.0	0.0	14.4	83.2	0.2
	3 months	15.24	49.80	14,890	2.0	0.0	0.0	0.0	0.0	10.2	87.6	0.2

TABLE III

Blood Analysis (continued)

Group III. Caramel Coloring 30F-1 - - FEMALES

Rat No.	Time	Hemo-globin grams %	Hemato-crit vol. %	WBC	Differential Count							
					Eosino-philes	Baso-philes	Myelo-cytes	Juve-niles	Stab	Seg-mented	Lympho-cytes	Mono-cytes
51	Initial	15.0	42	14,250	1	0	0	0	0	10	89	0
	1 month	14.0	45	10,900	2	0	0	0	0	11	87	0
	2 months	15.0	46	14,150	4	0	0	0	0	10	86	0
	3 months	15.0	48	12,300	1	0	0	0	0	7	92	0
53	Initial	12.0	43	13,450	1	0	0	0	0	17	82	0
	1 month	14.8	48	14,450	5	0	0	0	0	19	76	0
	2 months	13.5	48	9,550	0	0	0	0	0	12	88	0
	3 months	14.5	49	13,550	6	0	0	0	0	26	68	0
55	Initial	14.0	48	13,900	1	0	0	0	0	19	79	1
	1 month	14.0	44	10,800	1	0	0	0	0	19	79	1
	2 months	15.2	49	9,800	4	0	0	0	0	19	77	0
	3 months	15.0	48	8,150	2	0	0	0	0	14	84	0
57	Initial	13.5	43	11,300	0	0	0	0	0	8	92	0
	1 month	14.7	42	8,050	1	0	0	0	0	27	72	0
	2 months	15.8	49	7,250	1	0	0	0	0	16	83	0
	3 months	15.5	46	12,900	4	0	0	0	0	30	66	0
59	Initial	13.3	48	18,150	1	0	0	0	0	10	89	0
	1 month	15.0	45	10,150	2	0	0	0	0	17	81	0
	2 months	14.0	47	12,800	1	0	0	0	0	23	76	0
	3 months	14.3	46	11,450	9	0	0	0	0	19	72	0
Average	Initial	13.16	44.80	11,340	0.8	0.0	0.0	0.0	0.0	12.8	86.2	0.2
	1 month	14.50	44.80	10,870	2.2	0.0	0.0	0.0	0.0	18.6	79.0	0.2
	2 months	14.70	47.80	10,710	2.0	0.0	0.0	0.0	0.0	16.0	82.0	0.0
	3 months	14.86	47.40	11,670	4.4	0.0	0.0	0.0	0.0	19.2	76.4	0.0

TABLE III

Blood Analysis (continued)

Group IV. Control - - MALES

Rat No.	Time	Hemoglobin grams %	Hematocrit vol. %	WBC	Differential Count							
					Eosinophiles	Basophiles	Myelocytes	Juveniles	Stab	Segmented	Lymphocytes	Monocytes
61	Initial	11.7	44	15,700	0	0	0	0	0	15	84	1
	1 month	15.0	48	15,150	4	0	0	0	0	15	81	0
	2 months	15.5	49	21,200	1	0	0	0	0	7	92	0
	3 months	16.0	51	12,200	2	0	0	0	0	18	80	0
63	Initial	13.0	48	10,400	0	0	0	0	0	5	95	0
	1 month	15.5	50	23,000	1	0	0	0	0	17	81	1
	2 months	15.0	48	20,650	2	0	0	0	0	13	85	0
	3 months	17.5	51	14,650	2	0	0	0	0	15	83	0
65	Initial	13.7	50	11,450	0	0	0	0	0	9	89	2
	1 month	15.5	54	20,700	0	0	0	0	0	6	94	0
	2 months	15.0	50	18,300	2	0	0	0	0	7	91	0
	3 months	16.0	51	14,200	1	0	0	0	0	9	89	1
67	Initial	12.5	46	14,200	0	0	0	0	0	12	88	0
	1 month	15.7	51	19,000	1	0	0	0	0	10	89	0
	2 months	15.5	48	18,100	2	1	0	0	0	12	84	1
	3 months	18.0	55	14,450	6	0	0	0	0	4	90	0
69	Initial	13.0	50	12,850	1	0	0	0	0	12	87	0
	1 month	16.0	50	16,850	4	0	0	0	0	21	75	0
	2 months	15.5	52	16,100	1	0	0	0	0	8	91	0
	3 months	16.8	53	15,150	1	0	0	0	0	14	85	0
Average	Initial	12.78	47.60	12,920	0.2	0.0	0.0	0.0	0.0	10.6	88.6	0.6
	1 month	15.54	50.60	18,940	2.0	0.0	0.0	0.0	0.0	13.8	84.0	0.2
	2 months	15.30	49.40	18,870	1.6	0.2	0.0	0.0	0.0	9.4	88.6	0.2
	3 months	16.86	52.20	14,130	2.4	0.0	0.0	0.0	0.0	12.0	85.4	0.2

TABLE III

Blood Analysis (continued)

Group IV. Control - - FEMALES

Rat No.	Time	Hemoglobin grams %	Hematocrit vol. %	WBC	Differential Count							
					Eosinophiles	Basophiles	Myelocytes	Juveniles	Stab	Segmented	Lymphocytes	Monocytes
71	Initial	13.0	44	12,700	1	0	0	0	0	5	94	0
	1 month	14.7	48	19,750	1	0	0	0	0	17	82	0
	2 months	14.0	47	20,300	7	0	0	0	0	12	81	0
	3 months	17.2	55	18,200	1	0	0	0	0	3	96	0
73	Initial	13.5	52	16,300	0	0	0	0	0	14	85	1
	1 month	15.5	53	18,550	1	0	0	0	0	10	89	0
	2 months	17.5	52	19,000	2	0	0	0	0	8	90	0
	3 months	17.0	53	13,150	0	0	0	0	0	12	88	0
75	Initial	15.0	49	11,700	1	0	0	0	0	16	83	0
	1 month	16.0	51	10,000	5	0	0	0	0	11	84	0
	2 months	16.5	49	9,150	2	0	0	0	0	18	79	1
	3 months	17.0	51	8,300	1	0	0	0	0	16	83	0
77	Initial	14.0	49	12,700	0	0	0	0	0	6	94	0
	1 month	13.5	46	17,200	3	0	0	0	0	16	81	0
	2 months	14.0	46	13,250	2	0	0	0	0	8	90	0
	3 months	15.0	49	11,750	2	0	0	0	0	14	84	0
79	Initial	12.8	43	13,600	1	0	0	0	0	10	89	0
	1 month	14.0	44	13,750	3	0	0	0	0	10	87	0
	2 months	13.5	45	15,150	1	0	0	0	0	11	87	1
	3 months	14.0	45	12,050	3	0	0	0	0	11	86	0
Average	Initial	13.66	47.40	13,400	0.6	0.0	0.0	0.0	0.0	10.2	89.0	0.2
	1 month	14.74	49.40	15,850	2.6	0.0	0.0	0.0	0.0	12.8	84.6	0.0
	2 months	15.10	47.80	15,370	2.8	0.0	0.0	0.0	0.0	11.4	85.4	0.4
	3 months	16.04	50.60	12,690	1.4	0.0	0.0	0.0	0.0	11.2	87.4	0.0

TABLE IV

Average Blood Picture - Males and Females

Time	Hemoglobin grams %	Hematocrit vol. %	WBC	Differential Count							
				Eosinophiles	Basophiles	Myelocytes	Juveniles	Stab	Segmented	Lymphocytes	Monocytes
Group I. Caramel Coloring 25A-1											
Initial	14.06	45.80	13,285	0.2	0.0	0.0	0.0	0.0	9.2	90.0	0.6
1 month	15.02	48.20	17,095	3.4	0.0	0.0	0.0	0.0	11.8	84.5	0.3
2 months	15.09	49.00	14,830	3.2	0.0	0.0	0.0	0.0	21.0	85.7	0.1
3 months	15.90	49.70	13,905	2.2	0.0	0.0	0.0	0.0	9.0	88.6	0.2
Group II. Caramel Coloring 30B-0											
Initial	15.30	47.10	16,705	0.5	0.0	0.0	0.0	0.0	13.1	86.1	0.3
1 month	14.83	48.50	14,820	2.7	0.0	0.0	0.0	0.0	17.7	79.5	0.1
2 months	14.84	48.30	14,580	2.7	0.0	0.0	0.0	0.1	18.0	79.1	0.1
3 months	15.76	50.40	14,410	3.8	0.0	0.0	0.0	0.0	20.5	75.6	0.1
Group III. Caramel Coloring 30F-1											
Initial	13.51	46.00	13,770	0.6	0.0	0.0	0.0	0.0	12.6	86.6	0.2
1 month	14.50	45.90	14,160	2.1	0.0	0.0	0.0	0.0	17.4	80.3	0.2
2 months	15.09	48.20	12,480	2.1	0.0	0.0	0.0	0.0	15.2	82.6	0.1
3 months	15.05	48.60	13,280	3.2	0.0	0.0	0.0	0.0	14.7	82.0	0.1
Group IV. Control											
Initial	13.22	47.50	13,160	0.4	0.0	0.0	0.0	0.0	10.4	88.8	0.4
1 month	15.14	49.50	17,395	2.3	0.0	0.0	0.0	0.0	13.3	84.3	0.1
2 months	15.20	48.60	17,120	2.2	0.1	0.0	0.0	0.0	10.4	87.0	0.3
3 months	16.45	51.40	13,410	1.9	0.0	0.0	0.0	0.0	11.6	86.4	0.1

TABLE V

Urine Analysis

Group	Rat No.	Sex	SUGAR				ALBUMIN				ACETONE			
			Initial	1 mo	2 mos	3 mos	Initial	1 mo	2 mos	3 mos	Initial	1 mo	2 mos	3 mos
I.	1	M	N*	N	N	N	N	N	N	N	N	N	N	N
25A-1	3	M	N	N	N	N	N	N	N	N	N	N	N	N
	5	M	N	N	N	N	N	N	N	N	N	N	N	N
	7	M	N	N	N	N	N	N	N	N	N	N	N	N
	9	M	N	N	N	N	N	N	N	N	N	N	N	N
	11	F	N	N	N	N	N	N	N	N	N	N	N	N
	13	F	N	N	N	N	N	N	N	N	N	N	N	N
	15	F	N	N	N	N	N	N	Tr**	N	N	N	N	N
	17	F	N	N	N	N	N	N	N	N	N	N	N	N
	19	F	N	N	N	N	N	N	Tr	N	N	N	N	N
II.	21	M	N	N	N	N	N	N	N	Tr	N	N	N	N
30B-0	23	M	N	N	N	N	N	N	N	N	N	N	N	N
	25	M	N	N	N	N	N	N	N	N	N	N	N	N
	27	M	N	N	N	N	Tr	N	N	N	N	N	N	N
	29	M	N	N	N	N	N	N	N	N	N	N	N	N
	31	F	N	N	N	N	N	N	Tr	N	N	N	N	N
	33	F	N	N	N	N	N	N	N	N	N	N	N	N
	35	F	N	N	N	N	N	N	Tr	N	N	N	N	N
	37	F	N	N	N	N	N	N	N	N	N	N	N	N
	39	F	N	N	N	N	N	N	N	N	N	N	N	N

* N - Negative findings

** Tr - trace

TABLE V

Urine Analysis (continued)

Group	Rat No.	Sex	SUGAR				ALBUMIN				ACETONE			
			Initial	1 mo	2 mos	3 mos	Initial	1 mo	2 mos	3 mos	Initial	1 mo	2 mos	3 mos
III.	41	M	N	N	N	N	N	N	Tr	N	N	N	N	N
30:1-1	43	M	N	N	N	N	N	N	Tr	N	N	N	N	N
	45	M	N	N	N	N	N	N	Tr	N	N	N	N	N
	47	M	N	N	N	N	N	N	Tr	N	N	N	N	N
	49	M	N	N	N	N	N	N	Tr	Tr	N	N	N	N
	51	F	N	N	N	N	N	N	N	N	N	N	N	N
	53	F	N	N	N	N	N	N	N	N	N	N	N	N
	55	F	N	N	N	N	N	N	N	N	N	N	N	N
	57	F	N	N	N	N	N	N	N	N	N	N	N	N
	59	F	N	N	N	N	N	N	N	N	N	N	N	N
IV.	61	M	N	N	N	N	Tr	N	N	N	N	N	N	N
Control	63	M	N	N	N	N	N	N	Tr	Tr	N	N	N	N
	65	M	N	N	N	N	N	N	Tr	N	N	N	N	N
	67	M	N	N	N	N	N	N	N	Tr	N	N	N	N
	69	M	N	N	N	N	N	N	N	Tr	N	N	N	N
	71	F	N	N	N	N	N	N	N	N	N	N	N	N
	73	F	N	N	N	N	N	N	N	N	N	N	N	N
	75	F	N	N	N	N	N	N	N	N	N	N	N	N
	77	F	N	N	N	N	N	N	N	N	N	N	N	N
	79	F	N	N	N	N	N	N	N	N	N	N	N	N

032

ACUTE ORAL TOXICITY STUDY IN RATS

ON

CARAMEL COLORINGS 25A-1, 30B-0, and 30F-1

FOR: Union Starch and Refining Company
Granite City, Illinois

Lot Nos. 25A-1, 30B-0, 30F-1

S.A. No. 79105

Peter Chacharonis, Ph.D.
Scientific Associates, Inc.
St. Louis, Missouri

April 5, 1963

SAMPLE DESCRIPTION

Sample Code	25A-1	30B-0	30F-1
Caramel Type	Single Strength Beverage	Single Strength Bakers	Single Strength Bakers
Solids-%	65	67	68
pH	2-3	3-4	2-4
Isoelectric Point, pH	< 2	< 4	< 4
Usage Level in Sodas, grams/liter	3-4	N.A.	N.A.
Carbohydrate Used	Starch Hydroly-zate	Starch Hydroly-zate	Molasses
Catalysts:			
Sulfuric Acid		X	
Ammonium Hydroxide	X	X	X
Sulfurous Acid or SO_2 Bisulfite Salts	X		X
Sodium Hydroxide	X		
Ammonium Sulfate			
Open or Closed Kettle	Closed	Closed	Open
Nitrogen Used Basis Finished Product, %	1.05	1.4	1.4
Sulfur Used Basis Finished Product, %	1.82	0.1	1.2
Reaction Temp., °F.	280	280	290
Reaction Time, Hrs.	2	1-1/4	1-1/2

Acute Oral Toxicity of Caramel Coloring

EXPERIMENTAL PROCEDURE:

Albino rats (Sprague-Dawley strain of both sexes) weighing 200 to 300 grams were fed measured single doses of a 75% aqueous solution of the samples by means of a catheter attached to a hypodermic syringe. Groups of five rats were fed at levels designed to blanket the toxicity range in a manner sufficient to supply data for the calculation of the LD_{50}. The animals were observed frequently on the day of dosing and daily thereafter for 14 days. Animals were autopsied and gross examination made. Results are shown in Tables I., II., and III.

RESULTS AND DISCUSSION:

TABLE I

Oral Mortality Data on Caramel Coloring 25A-1

Dose (cc/Kg)	Mort./No. Animals	% Mortality
20.0	0/5	0
22.5	0/5	0
25.0	0/5	0
27.5	0/5	0
30.0	0/5	0

The acute oral LD_{50} in rats was found to be greater than 30 cc per kilogram body weight. During the first 48 to 72 hours following tubing the animals at the higher dose levels displayed signs of definite discomfort and slight to severe diarrhea, which is not remarkable in light of the massive doses given. Autopsy of the sacrificed animals revealed no gross systemic damage.

TABLE II

Oral Mortality Data on Caramel Coloring 30B-0

Dose (cc/Kg)	Mort./No. Animals	% Mortality
20.0	0/5	0
22.5	0/5	0
25.0	0/5	0
27.5	0/5	0
30.0	0/5	0

The acute oral LD_{50} in rats was found to be greater than 30 cc per kilogram body weight. During the first 48 to 72 hours following tubing the animals at the higher dose levels displayed signs of definite discomfort and slight diarrhea to severe diarrhea. This is not remarkable in light of the massive doses given. Autopsy of the sacrificed animals revealed no systemic damage.

TABLE III

Oral Mortality Data on Caramel Coloring 30F-1

Dose (cc/Kg)	Mort./No. Animals	% Mortality
20.0	0/5	0
22.5	0/5	0
25.0	1/5	20
27.5	0/5	0
30.0	1/5	20

The acute oral LD_{50} in rats was found to be greater than 30 cc per kilogram body weight. During the first 48 to 72 hours following tubing the animals displayed signs of definite discomfort and slight to severe diarrhea, which is not remarkable in light of the massive doses given. Death occurred within 24 hours in the case of the two animals which succumbed. Gross autopsy of these animals was not remarkable, with the exception of excessive coloration and pronounced distention of the gastrointestinal tract. Autopsy of the sacrificed animals at the end of the observation period was not remarkable.

SUMMARY:

1. The acute oral LD_{50} of Caramel Coloring 25A-1 in male and female albino rats was found to be greater than 30 cc/Kg body weight.

2. The acute oral LD_{50} of Caramel Coloring 30B-0 in male and female albino rats was found to be greater than 30 cc/Kg body weight.

3. The acute oral LD_{50} of Caramel Coloring 30F-1 in male and female albino rats was found to be greater than 30 cc/Kg body weight.

CODE OF FEDERAL REGULATIONS

TITLE 21

Parts 1 to 119

Revised as of January 1, 1969

CONTAINING A CODIFICATION OF DOCUMENTS OF GENERAL APPLICABILITY AND FUTURE EFFECT AS OF JANUARY 1, 1969

With Ancillaries

Published by the Office of the Federal Register, National Archives and Records Service
General Services Administration, as a Special Edition of the Federal Register
Pursuant to Section 11 of the Federal Register Act as Amended

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§ 8.301 Dried algae meal.

(a) *Identity.* The color additive dried algae meal is a dried mixture of algae cells (genus *Spongiococcum*, separated from its culture broth), molasses, corn-steep liquor, and a maximum of 0.3 percent ethoxyquin. The algae cells are produced by suitable fermentation, under controlled conditions, from a pure culture of the genus *Spongiococcum*.

(b) *Uses and restrictions.* The color additive dried algae meal may be safely used in chicken feed in accordance with the following prescribed conditions:

(1) The color additive is used to enhance the yellow color of chicken skin and eggs.

(2) The quantity of the color additive incorporated in the feed is such that the finished feed:

(i) Is supplemented sufficiently with xanthophyll and associated carotenoids so as to accomplish the intended effect described in subparagraph (1) of this paragraph; and

(ii) Meets the tolerance limitation for ethoxyquin in animal feed prescribed in § 121.202 of this chapter.

(c) *Labeling.* The label of the color additives and any premixes prepared therefrom shall bear in addition to the information required by § 8.32:

(1) A statement of the concentrations of xanthophyll and ethoxyquin contained therein.

(2) Adequate directions to provide a final product complying with the limitations prescribed in paragraph (b) of this section.

(d) *Exemption from certification.* Certification of this color additive is not necessary for the protection of the public health and therefore batches thereof are exempt from the certification requirements of section 706(c) of the act.

[33 F.R. 8813, June 18, 1968]

§ 8.302 β-Apo-8'-carotenal.

(a) *Identity.* (1) The color additive is β-apo-8'-carotenal.

(2) Color additive mixtures for food use made with β-apo-8'-carotenal may contain only diluents that are suitable and that are listed in this subpart as safe in color additive mixtures for coloring foods.

(b) *Specifications.* β-Apo-8'-carotenal shall conform to the following specifications:

Physical state, solid.
1 percent solution in chloroform, clear.
Melting point (decomposition), 136° C.–140° C. (corrected).
Loss of weight on drying, not more than 0.2 percent.
Residue on ignition, not more than 0.2 percent.
Lead (as Pb), not more than 10 parts per million.
Arsenic (as As), not more than 1 part per million.
Assay (spectrophotometric), 96–101 percent.

(c) *Uses and restrictions.* The color additive β-apo-8'-carotenal may be safely used for coloring foods generally, subject to the following restrictions:

(1) The quantity of β-apo-8'-carotenal does not exceed 15 milligrams per pound of solid or semisolid food or 15 milligrams per pint of liquid food.

(2) It may not be used to color foods for which standards of identity have been promulgated under section 401 of the act unless added color is authorized by such standards.

(d) *Labeling.* The label of the color additive and any mixtures prepared therefrom and intended solely or in part for coloring purposes shall conform to the requirements of § 8.32.

(e) *Exemption from certification.* Certification of this color additive is not necessary for the protection of the public health and therefore batches thereof are exempt from the certification requirements of section 706(c) of the act.

[33 F.R. 8813, June 18, 1968]

§ 8.303 Caramel.

(a) *Identity.* (1) The color additive caramel is the dark-brown liquid or solid material resulting from the carefully controlled heat treatment of the following food-grade carbohydrates:

Dextrose.
Invert sugar.
Lactose.
Malt sirup.
Molasses.
Starch hydrolysates and fractions thereof.
Sucrose.

(2) The food-grade acids, alkalis, and salts listed in this subparagraph may be employed to assist caramelization, in amounts consistent with good manufacturing practice.

(i) Acids:

Acetic acid.
Citric acid.
Phosphoric acid.
Sulfuric acid.
Sulfurous acid.

(ii) Alkalis:

Ammonium hydroxide.
Calcium hydroxide U.S.P.
Potassium hydroxide.
Sodium hydroxide.

(iii) Salts: Ammonium, sodium, or potassium carbonate, bicarbonate, phosphate (including dibasic phosphate and monobasic phosphate), sulfate, and sulfite.

(3) Polyglycerol esters of fatty acids, identified in § 121.1120 of this chapter, may be used as antifoaming agents in amounts not greater than that required to produce the intended effect.

(4) Color additive mixtures for food use made with caramel may contain only diluents that are suitable and that are listed in this subpart as safe in color additive mixtures for coloring foods.

(b) *Specifications.* Caramel shall conform to the following specifications:

Lead (as Pb), not more than 10 parts per million.
Arsenic (as As), not more than 3 parts per million.
Mercury (as Hg), not more than 0.1 part per million.

(c) *Uses and restrictions.* Caramel may be safely used for coloring foods generally, in amounts consistent with good manufacturing practice, except that it may not be used to color foods for which standards of identity have been promulgated under section 401 of the act unless added color is authorized by such standards.

(d) *Labeling.* The label of the color additive and any mixtures prepared therefrom and intended solely or in part for coloring purposes shall conform to the requirements of § 8.32.

(e) *Exemption from certification.* Certification of this color additive is not necessary for the protection of the public health and therefore batches thereof are exempt from the certification requirements of section 706(c) of the act.

[33 F.R. 8813, June 18, 1968]

§ 8.304 β-Carotene.

(a) *Identity.* (1) The color additive is β-carotene prepared synthetically or obtained from natural sources.

(2) Color additive mixtures for food use made with β-carotene may contain only diluents that are suitable and that are listed in this subpart as safe in color additive mixtures for coloring foods.

(b) *Specifications.* β-carotene shall conform to the following specifications:

Physical state, solid.
1 percent solution in chloroform, clear.
Loss of weight on drying, not more than 0.2 percent.
Residue on ignition, not more than 0.2 percent.
Lead (as Pb), not more than 10 parts per million.
Arsenic (as As), not more than 3 parts per million.
Assay (spectrophotometric), 96–101 percent.

(c) *Uses and restrictions.* The color additive β-carotene may be safely used for coloring foods generally, in amounts consistent with good manufacturing practice, except that it may not be used to color those foods for which standards of identity have been promulgated under section 401 of the act unless added color is authorized by such standards.

(d) *Labeling.* The label of the color additive and any mixtures prepared therefrom and intended solely or in part for coloring purposes shall conform to the requirements of § 8.32.

(e) *Exemption from certification.* Certification of this color additive is not necessary for the protection of the public health and therefore batches thereof are exempt from the certification requirements of section 706(c) of the act.

[33 F.R. 8814, June 18, 1968]

§ 8.305 Annatto extract.

(a) *Identity.* (1) The color additive annatto extract is an extract prepared from annatto seed, *Bixa orellana* L., using any one or an appropriate combination of the food-grade extractants listed in subdivisions (i) and (ii) of this subparagraph:

(i) Alkaline aqueous solution, alkaline propylene glycol, ethyl alcohol or alkaline solutions thereof, edible vegetable oils or fats, mono- and diglycerides from the glycerolysis of edible vegetable oils or fats. The alkaline alcohol or aqueous extracts may be treated with food-grade acids to precipitate annatto pigments, which are separated from the liquid and dried, with or without intermediate recrystallization, using the solvents listed under subdivision (ii) of this subparagraph. Food-grade alkalis or carbonates may be added to adjust alkalinity.

(ii) Acetone, ethylene dichloride, hexane, isopropyl alcohol, methyl alcohol, methylene chloride, trichloroethylene.

CHEMICALS USED IN FOOD PROCESSING

Food Protection Committee

Food and Nutrition Board

National Academy of Sciences—National Research Council

Publication 1274

National Academy of Sciences—National Research Council

Washington, D. C.

1965

d-CAMPHOR (cont'd)

Natural food occurrence:
Basil, ho oil, iva herb extract, zedoary-bark extract

Foods in which used:	Approx. Avg Maximum ppm
Ice cream, ices	0.10
Candy	25, 1.1
Baked goods	11
Condiments	20

CAPRALDEHYDE
(See Decanal)

CAPRIC ACID
(See Decanoic acid)

CAPRIC ALDEHYDE
(See Decanal)

CAPRINALDEHYDE
(See Decanal)

CAPROALDEHYDE
(See Hexanal)

CAPROIC ACID
(See Hexanoic acid)

CAPROIC ALDEHYDE
(See Hexanal)

CAPRONIC ETHER ABSOLUTE
(See Ethyl hexanoate)

CAPRYL ALCOHOL
(See 1-Octanol)

CAPRYL ALCOHOL (Secondary)
(See 2-Octanol)

CAPRYLALDEHYDE
(See Octanal)

CAPRYLIC ACID
(See Octanoic acid)

CAPRYLIC ALCOHOL
(See 1-Octanol)

CAPRYLIC ALDEHYDE
(See Octanal)

CARAMEL COLOR

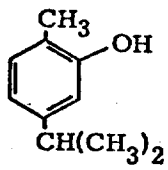
Derivation:
Heating and caramelizing of sugar

Flavors in which used:
Strawberry, butter, butterscotch, caramel, chocolate, cocoa, cola, fruit, cherry, grape, birch beer, liquor, rum, brandy, maple, meat, nut, black walnut, walnut, root beer, spice, ginger, ginger ale, vanilla, cream soda

Foods in which used:	Approx. Avg Maximum ppm
Beverages	2,200
Ice cream, ices	590
Candy	180
Baked goods	220
Sirups	2,800
Meats	2,100

CARVACROL

Chemical formula:



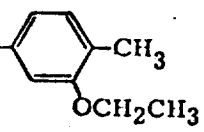
Flavors in which used:
Citrus, fruit, mint, spice

Natural food occurrence:
Dittany of Crete oil, oregano, lovage oil, marjoram, origanum oil, savory

Foods in which used:	Approx. Avg Maximum ppm
Beverages	26
Ice cream, ices	34
Candy	92
Baked goods	120
Condiments	37

CARVACRYL ETHYL ETHER
2-Ethoxy-p-cymene

Chemical formula: $(CH_3)_2CH$-



Flavors in which used:
Spices

SUMMARY REPORT

No. 1

on

TOXICITY OF CARAMEL COLOR PRODUCTS (102B, 103B, 104B, 107B, 108B, 109B and 110B)

to

UNION STARCH AND REFINING CO., INC.

by

W. L. Foote, R. F. Robinson, and R. S. Davidson

May 1, 1958

BATTELLE MEMORIAL INSTITUTE
505 King Avenue
Columbus 1, Ohio

SAMPLE DESCRIPTION

Sample Code	102B	103B	104B	107B	108B	109B	110B
Caramel Type	Single Strength Beverage	Single Strength Beverage	Single Strength Beverage	(1)	(2)	(3)	Double Strength Beverage
Solids-%	70	70	70	49	82	82	72
pH	2-4	2-3	2-3	2.5-3.5	3.5-4.5	3.5-4.5	2.9
Isoelectric Point, pH	< 4	<2	< 2	< 2			< 2
Usage Level in Sodas, grams/liter	3-4	3-4	3-4	1-3	N.A.	N.A.	1-3
Carbohydrate Used	Starch Hydroly-zate	Starch Hydroly-zate	Starch Hydroly-zate	Starch Hydroly-zate	Starch Hydroly-zate	Starch Hydroly-zate	Starch Hydroly-zate
Catalysts:							
Sulfuric Acid			X	X		X	
Ammonium Hydroxide	X	X	X	X			X
Sulfurous Acid or SO_2		X					
Bisulfite Salts	X		X	X			X
Sodium Hydroxide	X					X	
Ammonium Sulfate			X	X			X
Open or Closed Kettle	Closed	Open	Closed	Closed Alcohol Extracted	Closed	Closed	Closed
Nitrogen Used Basis Finished Product, %	.59	0.8	.92	.40		--	1.36
Sulfur Used Basis Finished Product, %	.88	1.0	1.13	.53		.09	4.95
Reaction Temp., °F.	280	270	280	280		235	295
Reaction Time, Hrs.	1	2	1-1/2	1-1/2		2/3	2-1/4

(1) 800 Caramel Color was produced by solvent extraction of color bodies from a direct burn caramel color. The reaction conditions and catalyst usage figure shown representsthose for production of the direct burn caramel.

(2) 800 Residual Sugar was produced as outline above for 800 Caramel Color. The residual sugar, representing approximately two-thirds of the solids of the original direct burn caramel, can be recirculated for reburning.

(3) Acid-Treated Sugar, an intermediate in the manufacture of some caramel color types.

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TOXICITY OF CARAMEL COLOR PRODUCTS

by

W. L. Foote, R. F. Robinson, and R. S. Davidson

INTRODUCTION

A chemical or mixture of chemicals of known or reproducible composition used in addition to the basic foodstuff and present in the foodstuff as purchased are termed chemical additives. Caramel color products undoubtedly must be included in this classification. Therefore, it is desirable to investigate their biological effects and establish that they will not harm the consumer.

Toxicity studies are usually guided by a knowledge of the chemical and physical properties of the substance and the effect of substances having similar properties or structure. Upon this information studies are designed to emphasize any suspected potential of the substance for injury, thus providing as stringent a test as possible. However, as the above information on caramel color products was not available, this toxicity study was necessarily designed around the anticipated levels and patterns of consumption.

SUMMARY

The acute oral toxicity (ED_{50}) of caramel color products could not be estimated as the maximum dosage that could be given did not present any symptoms of toxicity. However, man is generally considered to be ten times more sensitive than the rat; so, on the basis that the maximum

dosage given to rats was 2.3 milliters/kilogram, a man weighing 154 pounds who ingested 16.1 milliters of a caramel color product at one time would not be adversely affected. Thus, if the caramel were added to a beverage at a level of 0.10 per cent, a man weighing 154 pounds could theoretically consume 16 liters (about 17 quarts) of the beverage at one time without being adversely affected due to the presence of the additive.

The ingestion of an average of 1.05ml/kg of caramel color products daily by rats over a 21-day period also showed no toxicity effects. Darkly stained fecal matter of varying degrees of softness and several instances of diarrhea was evident in all treated animals. However, a gross and microscopic pathological examination revealed no significant pathology. The rate of recovery from tarry stools was almost instantaneous after terminating the treatments. Interpretation of these results would indicate that a man weighing 154 pounds who ingested 7.3 ml of a caramel color product daily for a period of 21 days would not be adversely affected.

EXPERIMENTAL WORK

The acute and subacute oral toxicities were determined for seven caramel color products (designated as 102-B, 103-B, 104-B, 107-B, 108-B, 109-B, and 110-B). The procedures used in the evaluation of the toxicity of caramel colors follow the outline of general suggestions offered by officials of the Food and Drug Administration. Their recommendations appeared in "Procedures for the Appraisal of the Toxicity of Chemicals in Food, Drugs, and Cosmetics" by A. J. Lehman et al, Food, Drug, Cosmetic Law Journal, October, 1955 (10:10, pp 679-748).

The detailed descriptions of all the work performed on this project are contained in Battelle Record Books Nos. 14261 and 14473.

Facilities

The small animal laboratory used in this program was a well ventilated room with an area of approximately 350 square feet. The rats were individually housed in 1-inch wire mesh cages measuring 9-5/8 x 7 x 7 inches. These cages were suspended in sets of 60 in "paper-roll" batteries. Each cage had its own water supply. Rats used in the studies were fed Rockland mouse diet in pellet form from wire mesh feeders inside the cage. There was an unlimited supply of feed and water available to the rats at all times. Since the cages were suspended in batteries, all excreta dropped through the mesh onto the paper-lined trays which were cleaned daily.

Acute Oral Toxicity

The first phase of the examination of the toxic properties of caramel color products was to establish the acute oral toxicity. This toxicity is often expressed as the ED_{50}, that dose which, upon a single oral administration, affects 50 per cent of the treated animals. Young male and female albino rats of the Sprague-Dawley strain were used in both the acute and sub-acute oral toxicity studies.

In a case where there are no data on the biological effects of a material, the first step is to get an estimation of the toxic dose. This is done by administering doses spaced at 0.1 log intervals to groups of ten animals, 5 male and 5 female. The information from this test is also used in selecting dosages for the sub-acute oral toxicity determinations.

The acute exploratory doses used in this study were 0.501, 0.631, 0.794, and 1.0 milliters. The caramel color products were dissolved in distilled water to give the desired dosages and these were administered to the rats by stomach tube. A 10-ml hypodermic syringe and a 4-inch section of rubber catheter were used in the intubations. Each rat received 1 ml of the solution at the desired dosage level. This is the maximum safe dosage usually administered by intubation. The average weight of the rats in each group was 239.8 g, therefore the animals receiving 1.0 ml of the study materials actually received an approximate dosage of 2.30 ml/kg.

The animals were observed daily for two weeks following treatment. There was no indication of toxicity in any of the treated or control animals. At the termination of the observation period, 1 male and 1 female from the highest dosage level of each group were autopsied and examined for gross pathology. There was no evidence of toxicity to be noted in any of the animals, during the study or upon gross examination.

On the basis of information gleaned from this phase of the program alone, it can be concluded, as man is generally considered to be ten times more sentitive than the rat, that a man weighing 154 pounds who ingested not more than 16.1 milliters of a caramel color product at one time would not be affected.

Sub-Acute Oral Toxicity

In the determination of sub-acute oral toxicity, as in the acute phase, it is necessary to establish an approximation of the toxic level

of the material being investigated before full-scale tests are run. This is done on the basis of information gleaned in the acute toxicity trial.

By examining the information obtained from the acute work, it was decided that the maximum quantity of each of the materials would be administered, i.e., 1 ml of the undiluted products. Groups of 5 male and 5 female albino Sprague-Dawley rats were established by assigning the animals on the basis of weight in such amanner that each group had the same average weight and the same range of weight. The feed for each animal was weighed out and the amount recorded. Records were kept of weight changes, food consumption, and grossly detectable symptoms as well as of mortality.

The maximum tolerated dosage of each of the materials to be given was 1 ml of the concentrated material daily for 21 days. Great difficulty was experienced in intubating all of the materials as the viscosity of each was sufficiently great enough to inhibit the passage of air into the lungs when any of the material was deposited in the airway. An active gag reflex was apparent in almost all animals intubated. An attempt was made to irrigate and aspirate the oral cavity of all animals thus affected. However, in a number of cases this procedure did not effectively clear the airway and within a few minutes the animals died of suffocation. Animals from each group were autopsied and it was evident that death was due to the mechanical blockage of the airway. Mortality averaged from 1-4 animals in all groups except the control and 103-B groups. The 110-B group was the highest with 40 per cent mortality. Animals which survived were normal by the following morning.

DXV

Replacement animals were ordered from the same source and intubations were immediately resumed with the materials diluted 1:1 with distilled water. Although this reduced the daily dosage of the concentrated material to 0.5 ml per animal, it allowed trouble-free intubations by decreasing the viscosity of the materials. At the end of the second day of intubations, two incidents of diarrhea were noted in the 109-B group and one animal in the 102-B, 104-B, and 108-B groups each had soft tarry stools. By the end of the first week, all treated animals had tarry stools with some degree of softness.

One rat died during the first week of treatment due to a ruptured esophagus inflicted during intubation. There were no more mortalities during the study. The tarry fecal matter which was evident in all treated animals disappeared immediately upon termination of treatments. There were no symptoms in the animals of the control.

The average total weight changes and food consumption for each group and for the males and females in each group are shown in Table 1. No significant differences were found in the various groups.

A pathological examination was made of the organs and digestive tract from two male and two female rats from each group, including the control group. The evaluations were based upon gross and microscopic examinations from the control rats. The pathologist reported that there was some congestion of the spleen and kidneys in the 108-B, 109-B, and 110-B groups. However, it was concluded that the caramel color products in the dosages administered did not contribute to any pathology which would indicate the presence of a significant toxic agent.

DISCUSSION

The procedures used in the acute and sub-acute toxicity studies follow the recommendations of the Pure Food and Drug Administration. The preliminary screening trials were necessary as no preliminary data could be provided by the Sponsor.

Albino rats of the Sprague-Dawley strain were used in all the oral studies. The difference in the susceptibility of various strains of rats to toxicants has been of great interest to workers in this field. It is the opinion of some breeders of laboratory animals that Sprague-Dawley rats are particularly sensitive and, therefore, excellent animals to be used on toxicity evaluations.

This program has been a preliminary toxicological research effort designed to give an approximate value of the effects of caramel color products and a background for further evaluation of the products as intentional chemical additives for the purpose of imparting desirable qualities to foods and beverages. The organs and digestive tract from one male and one female rat from each group have been preserved in labeled bottles containing a 10 per cent formalin solution, and are available to the Sponsor at any time.

WLF:RFR:RSD/rn

TABLE 1. PER CENT INCREASES IN BODY WEIGHT AND AVERAGE TOTAL FOOD CONSUMPTION - SUB-ACUTE ORAL TOXICITY OF CARAMEL COLOR PRODUCTS

Group	Number of Rats	Initial Weight, grams	Final Weight, grams	Weight Gain, grams	Weight Gain, per cent	Total Average Food Consumption During 5-Week Trial, grams
Control						
Total	10	176.0	286.12	110.12	62.57	768.72
Males	5	175.5	339.0	163.5	93.18	884.9
Females	5	177.7	233.3	55.6	31.29	652.0
102-B						
Total	10	177.4	288.25	110.85	62.48	758.24
Males	5	175.4	324.0	148.6	84.72	906.55
Females	5	179.3	252.5	73.2	40.83	652.7
103-B						
Total	10	173.8	281.7	107.9	62.08	725.17
Males	5	170.7	325.2	154.5	90.51	814.90
Females	5	176.9	238.3	61.4	34.19	635.4
104-B						
Total	10	171.85	279.5	107.65	62.64	695.9
Males	5	166.4	311.4	145.0	87.14	736.9
Females	5	177.3	237.17	59.78	33.72	649.3
107-B						
Total	10	175.85	288.2	112.35	63.89	746.43
Males	5	175.5	325.9	150.4	85.70	768.0
Females	5	176.2	238.0	61.8	35.07	694.1
108-B						
Total	10	174.6	283.5	108.9	62.37	749.57
Males	5	175.6	328.5	152.4	86.78	853.4
Females	5	173.6	238.5	64.9	37.38	645.6
109-B						
Total	10	174.25	268.87	94.62	54.30	716.0
Males	5	171.1	312.0	140.9	82.35	803.0
Females	5	177.4	225.7	48.3	27.23	633.0
110-B						
Total	10	172.7	279.87	107.17	62.06	707.8
Males	5	168.8	322.0	153.2	90.78	772.5
Females	5	176.6	237.7	61.1	34.60	643.2

Battelle Memorial Research Project April 11, 1958

Mr. Wendell Foote, Battelle Memorial Institute, 505 King Avenue, Columbus, Ohio

Tissues - RAT

RAT TISSUES - delivered by Mr. Foote, March 21, 1958

GROSS

Our Number

15025 Labeled Control, #3, female; 2 kidneys, liver, spleen, heart, stomach, and colon, sectioned for microscopic examination.

15026 Labeled Control, #8, male; 2 kidneys, liver, spleen, heart, stomach, colon and small intestine, sectioned for microscopic examination.

15027 Labeled #102-B, #13, female; 2 kidneys, liver, spleen, heart, stomach, and small intestine, sectioned for microscopic examination.

15028 Labeled #102-B, #18, male; 2 kidneys, liver spleen, heart, stomach, colon and small intestine, sectioned for microscopic examination.

15029 Labeled #103-B, #23, female; 2 kidneys, liver, spleen, heart, stomach, colon and small intestine, sectioned for microscopic examination.

15030 Labeled #103-B, #28, male; 2 kidneys, liver, spleen, heart, stomach, colon and small intestine, sectioned for microscopic examination.

NOTE: Up to this point there is no gross pathology noted in any of the organs.

15031 Labeled #104-B, #32, female, 2 kidneys, liver, spleen, heart, stomach, colon and small intestine, sectioned for microscopic examination.

15032 Labeled #104-B, #38, male; 2 kidneys, liver, spleen, heart, stomach, colon and small intestine, sectioned for microscopic examination.

15033 Labeled #107-B, #43, female; 2 kidneys, liver, spleen, heart, stomach, colon and small intestine, sectioned for microscopic examination.

15034 Labeled #107-B, #48, male; 2 kidneys, liver, spleen, heart, stomach, colon and small intestine, sectioned for microscopic examination.

H.L. Reinhart, M.D.

Battelle Project 4-11-58

Mr. Foote

Rat Tissues

GROSS (cont)

Our Number

15035 Labeled #108-B, #53, female; 2 kidneys, liver, spleen, heart, stomach, colon and small intestine, sectioned for microscopic examination.

15036 Labeled #108-B, #58, male; 2 kidneys, liver, spleen, heart, stomach, colon and small intestine, sectioned for microscopic examination.

15037 Labeled #109-B, #123, female; 2 kidneys, liver, spleen, heart, stomach, colon and small intestine, sectioned for microscopic examination.

15038 Labeled #109-B, #128, male; 2 kidneys, liver, spleen, heart, stomach, colon and small intestine, sectioned for microscopic examination.

15039 Labeled #110-B, #133, female; 2 kidneys, liver, spleen, heart, stomach, colon and small intestine, sectioned for microscopic examination.

15040 Labeled #110-B, #138, male; 2 kidneys, liver, spleen, heart, stomach, colon and small intestine, sectioned for microscopic examination.

MICROSCOPIC

15025 Microscopic examination of liver, spleen, kidney, heart, stomach and colon; no significant pathology.

15026 Microscopic examination of lung, liver, spleen, colon, kidney, stomach; no significant pathology.

15027 Microscopic examination of liver, kidney, spleen, heart, colon, stomach, small intestine; no significant pathology.

15028 Microscopic examination of liver, stomach, heart, colon, small intestine, pancreas; no significant pathology.
Spleen; moderate congestion.
Kidneys; moderate congestion.

H.L. Reinhart, M.D.

Battelle Project 4-11-58

Mr. Foote

Rat Tissues

MICROSCOPIC (cont)

Our Number

15029 Microscopic examination of liver, spleen, kidney, pancreas, colon, heart and stomach; no significant pathology.

15030 Microscopic examination of heart, kidney, colon, liver, spleen, small intestine, stomach; no significant pathology.

15031 Microscopic examination of pancreas, kidney, heart, liver, stomach, colon, spleen, and small intestine; no significant pathology.

15032 Microscopic examination of kidney, heart, colon, small intestine, spleen, stomach, liver; no significant pathology.

15033 Microscopic examination of liver, spleen, kidney, colon, small intestine, heart, pancreas, stomach; no significant pathology.

15034 Microscopic examination of liver, spleen, stomach, kidneys, adrenal glands, pancreas, small intestine, heart; no significant pathology.
There is congestion of the spleen with some hemolysis of R.B.C. and blood pigment.
Kidneys present moderate congestion of tubular portions with some blood pigmentation present.
No noteworthy pathology.

15035 Microscopic examination of heart, kidneys, stomach, small intestine, spleen, liver; no significant pathology.
There is moderate congestion of spleen and slight congestion of kidneys.
Tissues are not otherwise remarkable.

15036 – Microscopic examination of heart muscle, small intestine, spleen, kidneys, stomach and liver; no significant pathology.
Moderate congestion of the spleen.
Not otherwise remarkable.

H.L. Reinhart, M.D.

Battelle Project 4-11-58

Mr. Foote

Rat Tissues

MICROSCOPIC (cont)

Our Number

15037 Microscopic examination of liver, pancreas, stomach, spleen, kidneys, small intestine, adrenals, heart; no significant pathology.
Moderate congestion of spleen and kidneys.
Not otherwise remarkable.

15038 Microscopic examination of stomach, pancreas, spleen, heart, kidneys, liver; no significant pathology.
Moderate congestion of spleen and kidneys.
Not otherwise remarkable.

15039 Microscopic examination of kidneys, stomach, spleen, pancreas, heart, liver; no significant pathology.
Slight congestion of spleen.
Not otherwise remarkable.

15040 Microscopic examination of heart, kidneys, adrenals, stomach, spleen, small intestine, liver; no significant pathology.
Moderate congestion of liver, kidneys, and spleen.
Not otherwise remarkable.

No significant pathology to date!
Report will be continued as soon as remaining slides for microscopic examination of organs from animals delivered to me April 8, 1958, are available.

H.L. Reinhart, M.D.

Battelle Memorial Research Project April 19, 1958

Mr. Wendell L. Foote, Battelle Memorial Institute, 505 King Avenue, Columbus, Ohio

Rat ~~and Guinea Pig~~ Tissues

RAT TISSUES - Rec'd April 4, 1958; delivered by Mr. Wendell Foote

GROSS

Our Number	
15041	Labeled Control, #2, male; kidneys and liver, sectioned for microscopic examination. Inspection of stomach, lung, spleen and intestinal tract reveals no gross evidence of pathology.
15042	Labeled Control, #7, female; kidneys, liver, heart, sectioned for microscopic examination. Inspection of stomach, lung, spleen and intestinal tract reveals no gross evidence of pathology.
15043	Labeled #102-B, #12, male; kidneys, heart and liver, sectioned for microscopic examinimination. Inspection of stomach, lung, spleen and intestinal tract reveals no gross evidence of pathology.
15044	Labeled #102-B, #17, female, kidneys, heart and liver, sectioned for microscopic examination. Inspection of stomach, lung, spleen and intestinal tract reveals no gross evidence of pathology.
15045	Labeled #103-B, #22, male; kidneys, heart and liver, sectioned for microscopic examination. Inspection of stomach, lung, spleen and intestinal tract reveals no gross evidence of pathology.
15046	Labeled #103-B, #26, female; kidneys, liver and heart, sectioned for microscopic examination. The lungs are small and somewhat congested, and are sectioned for microscopic examination. Inspection of stomach, spleen and intestinal tract reveals no gross evidence of pathology.
15047	Labeled #104-B, #26, male; kidneys, heart and liver, sectioned for microscopic examination. Inspection of stomach, lung, spleen and intestinal tract reveals no gross evidence of pathology.

H.L. Reinhart, M.D.

Battelle Project 4-19-58

Mr. Foote

Rat ~~and Guinea Pig~~ Tissues

GROSS (cont)

Our Number

15048 Labeled #104-B, #37, female; kidneys, heart and liver, sectioned for microscopic examination. Inspection of stomach, lung, spleen and intestinal tract reveals no gross evidence of pathology.

15049 Labeled #109-B, #122, male; kidneys, heart and liver, sectioned for microscopic examination. Inspection of stomach, lung, spleen and intestinal tract reveals no gross evidence of pathology.

15050 Labeled #109-B, #127, female; kidneys, heart and liver, sectioned for microscopic examination. Inspection of stomach, lung, spleen and intestinal tract reveals no gross evidence of pathology.

15051 Labeled #110-B, #134, male; kidneys, heart and liver, sectioned for microscopic examination. Inspection of stomach, lung, spleen and intestinal tract reveals no gross evidence of pathology.

15052 Labeled #110-B, #139, female; kidneys, heart, liver and lungs, sectioned for microscopic examination. Moderate congestion of lungs. Inspection of stomach, spleen and intestinal tract reveals no gross evidence of pathology.

15053 Labeled #107-B, #42, male; kidneys, heart and liver, sectioned for microscopic examination. Inspection of stomach, lung, spleen and intestinal tract reveals no gross evidence of pathology.

15054 Labeled #107-B, #47, female; kidneys, heart and liver, sectioned for microscopic examination. Inspection of stomach, lung, spleen and intestinal tract reveals no gross evidence of pathology.

15055 Labeled #108-B, #52, male; kidneys, heart and liver, sectioned for microscopic examination. Inspection of stomach, lung, spleen and intestinal tract reveals no gross evidence of pathology.

H. L. Reinhart, M.D.

Battelle Project 4-19-58

Mr. Foote

Rat ~~and Guinea Pig~~ Tissues

GROSS (cont)

Cur Number

15056 Labeled #108-B, #57, female; kidneys, heart and liver, sectioned for microscopic examination. Inspection of stomach, lung, spleen and intestinal tract reveals no gross evidence of pathology.

MICROSCOPIC

RATS

15041 Normal kidney and liver tissue.

15042 Heart, kidneys, adrenals and liver, present no noteworthy pathology.

15043 Heart, kidneys, adrenals and liver, present no noteworthy pathology.

15044 Kidneys, moderate congestion of vessels and moderate peri-pelvic infiltration of round cells.
Liver, adrenals and heart, present no noteworthy pathology.

15045 Heart, kidneys, adrenals and liver, present no noteworthy pathology.

15046 Heart, kidneys, adrenals and liver, present no noteworthy pathology.
Portion of lung included presents a moderate congestion of blood vessels

15047 Heart, kidneys and liver, present no noteworthy pathology.

15048 Heart, kidneys, adrenals and liver, present no noteworthy pathology.

15049 Heart, kidneys, adrenals and liver, present no noteworthy pathology.

H.L. Reinhart, M.D.

Battelle Project

4-19-58

Mr. Foote

Rat ~~and Guinea Pig~~ Tissues

MICROSCOPIC (cont)

Our Number

15050 Heart, kidneys, adrenals and liver, present no noteworthy pathology.

15051 Heart, kidneys, adrenals and liver, present no noteworthy pathology.

15052 Heart, kidneys, adrenals and liver, present no noteworthy pathology. Moderate congestion of lungs.

15053 Heart, kidneys, adrenals and liver, present no noteworthy pathology.

15054 Heart, kidneys, adrenals and liver, present no noteworthy pathology.

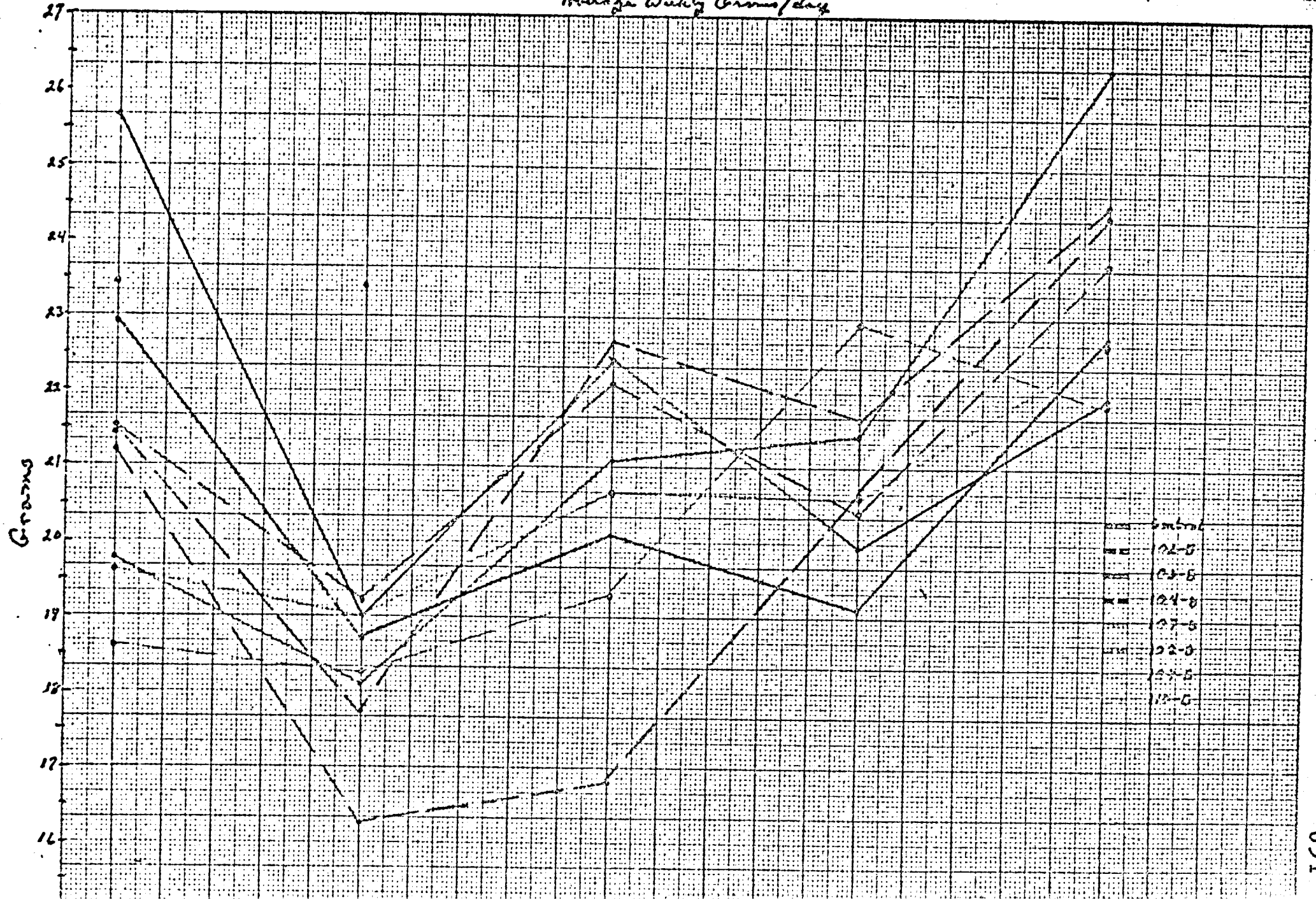
15055 Heart, kidneys, adrenals and liver, present no noteworthy pathology.

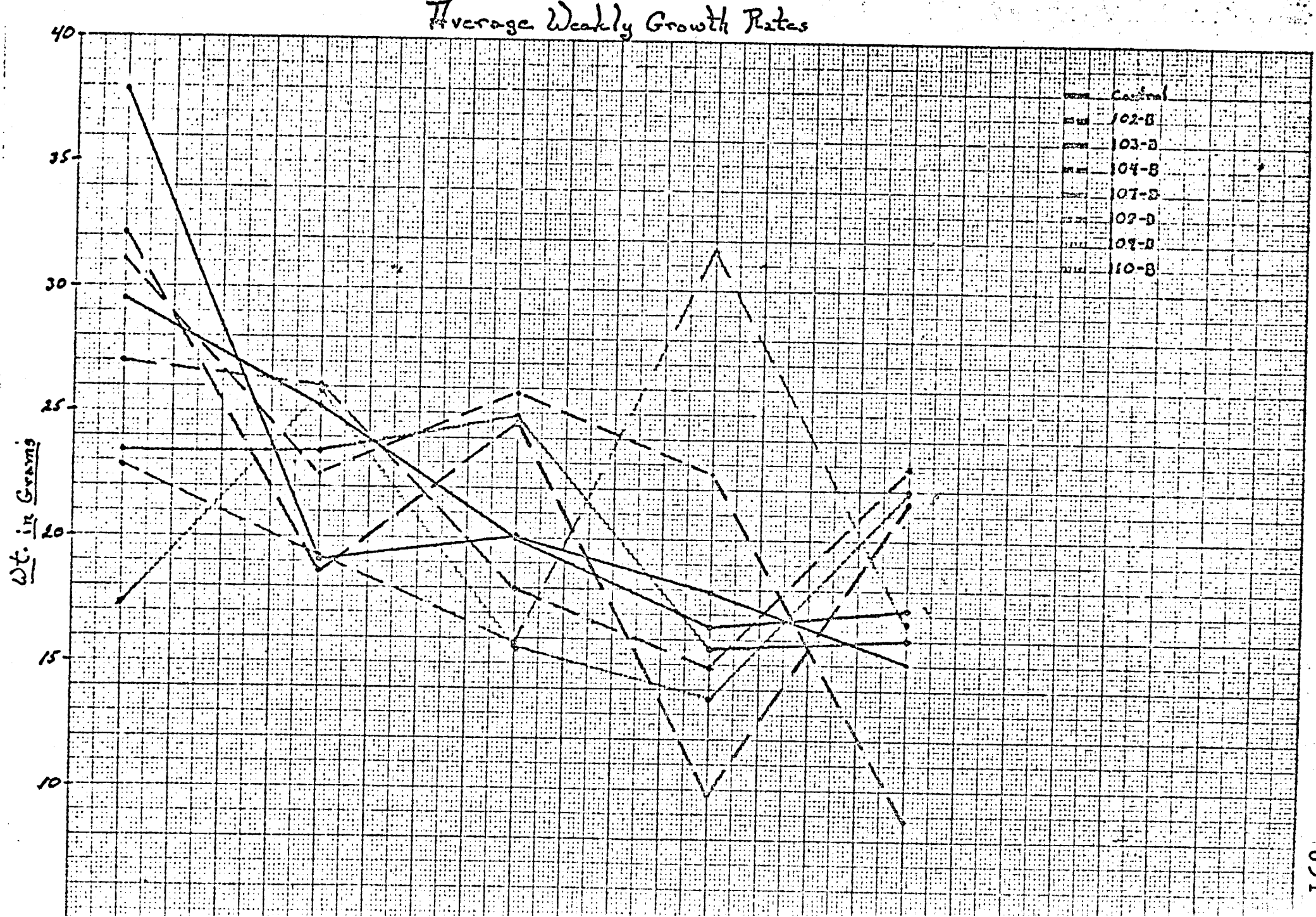
15056 Heart, kidneys, adrenals and liver, present no noteworthy pathology.

H.L. Reinhart, M.D.

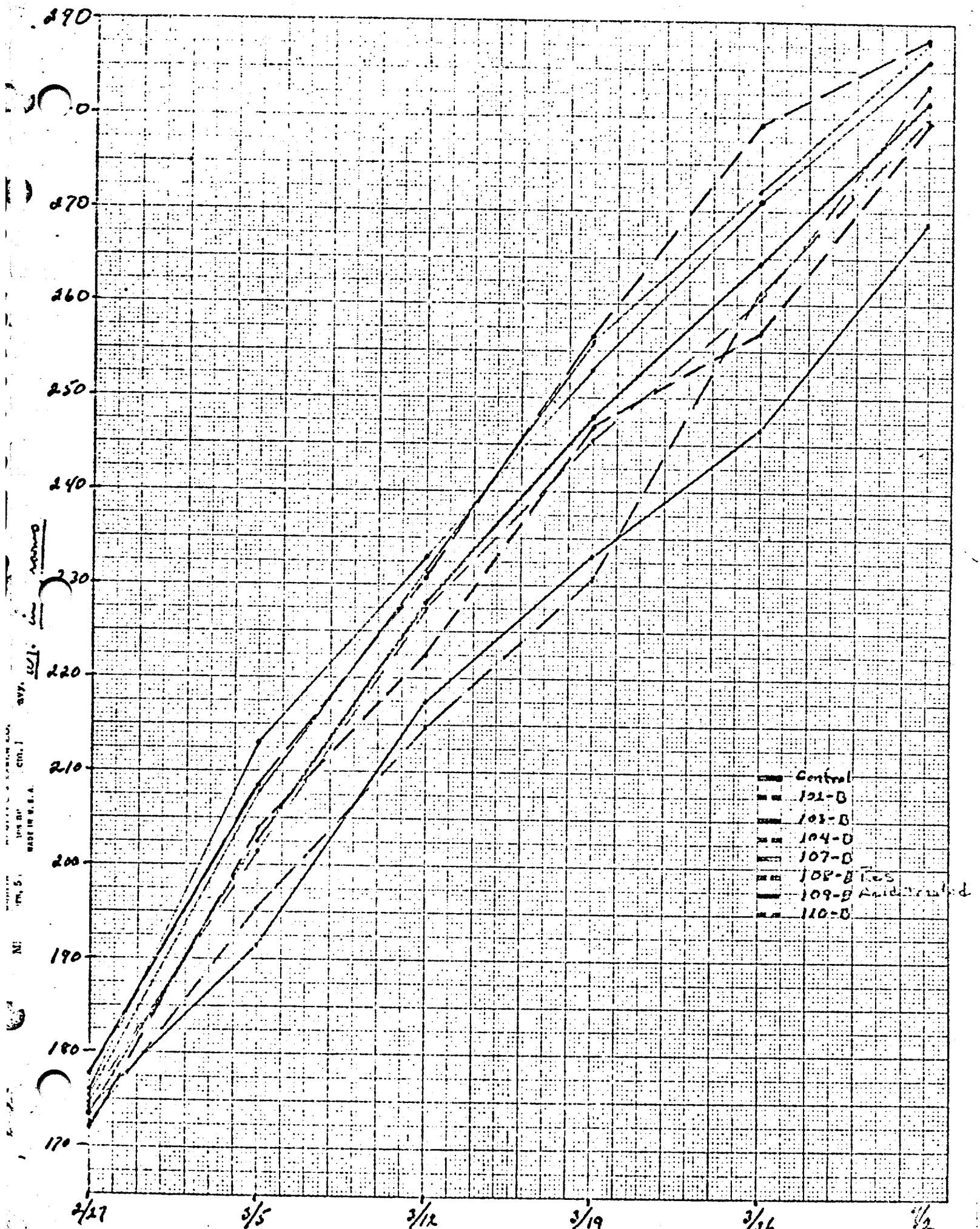
Weekly Food Consumption

Average Weekly Grams/day





Weekly Wt. Changes



A STUDY TO DETERMINE WHETHER OR NOT CARAMEL HAS ANY HARMFUL PHYSIOLOGICAL EFFECT.

I. 1951 II. 1958

by

John Haldi, Ph.D. and Winfrey Wynn, M.A.

Department of Physiology
School of Medicine
Emory University
Atlanta, Georgia

1951 and 1958

to

The Coca-Cola Company
Atlanta, Georgia

CARAMEL STUDIES

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1. Experiment on CPRCO; VE1, VE2 blend
2. Experiment on S-58; HX79, HX80 blend

Table

1. Experiment 73-X

Histological Report

NOTE

In this report, caramels* used are identified as follows:

1951

1. Regular caramel.
   Union Starch and Refining Co., open kettle.
   Carbohydrate: starch hydrolysates.
   Catalyst: ammonium hydroxide and sulfurous acid.
   Analysis: 0.8% N, 1.0% S.

2. S-4251; also called Hi-Test or High Acid Proof.
   Sethness Products Co., closed kettle.
   Carbohydrate: starch hydrolysates.
   Catalyst: ammonium hydroxide, sulfurous acid and sodium bisulfite.
   Analysis: 1.0% N, 0.8% S.

3. UL-14143; also called Hi-Test.
   Union Starch and Refining Co., closed kettle.
   Carbohydrate: starch hydrolysates.
   Catalyst: ammonium hydroxide and sulfurous acid.
   Analysis: 1.1% N, 1.2% S.

4. Dehydrated caramel:
   similar to 1, with 25% of moisture removed.

1958

5. CPRCO: 1:1 blend VE1 and VE2.
   Corn Products Refining Co., closed kettle.
   Carbohydrate: starch hydrolysates
   Catalyst: ammonium hydroxide, sulfurous acid and sodium bisulfite.
   Analysis: 0.7% N, 1.25% S.

6. S-58; 1:1 blend HX79 and HX80.
   Sethness Products Co., closed kettle.
   Carbohydrate: starch hydrolysate
   Catalyst: ammonium hydroxide, sulfurous acid and sodium bisulfite.
   Analysis: 0.7% N, 1.25% S.

*All test materials were so-called single strength caramels.

A STUDY TO DETERMINE WHETHER OR NOT CARAMEL HAS ANY HARMFUL PHYSIOLOGICAL ACTION

Summary of Results

The animals used in this experiment were albino rats of the Wistar strain.

Preliminary experiments showed that the rat will drink relatively large amounts of caramel dissolved in water. These experiments also indicated that caramel is not toxic.

These experiments were followed by carefully controlled experiments, which provide evidence to show that the single strength, acid proof caramel presently used in Coca Cola, and also two other varieties submitted to us, which are designated as S-4251 and U-L-14143, have no deleterious physiological action.

Growth, reproductivity, hemoglobin, red, white and differential cell counts of the blood and the histological structure of the liver, spleen, kidney, stomach, heart, lungs, pancreas, gonads, duodenum, small intestine and large intestine were not affected by the ingestion of large amounts of caramel over long periods of time. In some instances the animals ingested caramel for as long as 300 days which is approximately one-half the normal life span of a rat. The average intake on a very conservative basis may be taken as 2.5 gm. per day. Since the average weight was in the neighborhood of 200 gm., this intake was equivalent to about 5 gm. per pound of body weight. If these figures can be applied to man, these experiments indicate that an intake of about a pound of caramel per day by a child weighing 80 pounds or two pounds per day by an adult man weighing 160 pounds would of itself produce no harmful physiological effects.

Winifred Elgin

John Hall

CARAMEL STUDIES

REPORT ON EXPERIMENTS ON THE INGESTION OF CARAMEL BY ALBINO RATS

Experiment #71-Preliminary

Procedure

This experiment was a preliminary study to determine roughly what quantities of caramel the rats would ingest voluntarily.

Adult albinos were selected at random from the colony and were fed Purina Laboratory Chow ad libitum. 5 animals were offered 30 ml. 10% caramel solution made up in tap water each day; 5 more animals were offered 30 ml. 20% caramel solution each day. Observations were made on the general health of these animals, loss or gain in weight, and daily intake of caramel solution. This experiment was continued for 127 days after which time the animals were autopsied and tissues fixed for histological examination.

Results

All animals on both 10% and 20% caramel solution remained in good health throughout the time of the experiment, 127 days. The animals on 10% caramel drank on the average 27 ml. per day. This amounted to an average intake of 2.7 gm. caramel each day for a period of 127 days. The total amount of caramel ingested on the average was 338 gm. per rat. The animals on 20% caramel drank on the average 27 ml. per day. This amounted to an average intake of 5.4 gm. caramel each day. The total amount of caramel ingested on the average was 686 gm. per rat. These data are presented on table #B-1.

It was observed that the feces of all these animals were very dark in color, rather large in amount and occasionally there was some evidence of a very mild diarrhea. This observed diarrhea was so mild that it could in no way be considered harmful.

Reports on histological examination of the tissues of these animals were negative.

Experiment #71-A, First Generation Study

In view of the fact that the animals in Experiment #71-Preliminary were able to tolerate a large amount of caramel daily without harmful effects, it was decided to run a carefully controlled experiment using a 10% solution of caramel as the test material.

Procedure

60 albino rats were selected in litter mate pairs, shortly after weaning, their weights averaging from 50 to 65 gm. They were fed Purina Laboratory Chow ad libitum and the test animals were offered 30 ml. 10% caramel solution each day as their sole source of fluid; the control litter mates were given water to drink.

The experiment was divided into three parts. 10 rats were run as tests for 100 days and 10 rats were run as controls for the same period. 10 more were run as tests for 200 days and 10 as controls for the same period; also the same number of pairs were run for 300 days.

Observations were made on fluid intake, food intake, and caramel intake. Each animal was weighed at 10 day intervals to obtain growth curves. At the end of the 100, 200, and 300 day periods, the animals were autopsied and sections were made and fixed for histological examination. Blood was drawn from the tails of several test animals and several control animals at the end of 100 days, 200 days and 300 days. Hemoglobin, red cell count, white cell count, and differential counts were made.

Results

From figure #1 it may be seen that there was no appreciable difference in the rate of growth of the test and control animals.

The 100 day animals ingested 224 gm. caramel or on the average of 2.2 gm. each day; the 200 day animals ingested 468 gm. or an average of 2.4 gm. each day; the 300 day animals 755 gm or an average of 2.6 gm. each day. Table #B-2.

Histological examination of the tissues of these animals did not show any pathological changes which could be construed as having been caused by the ingestion of the caramel.

The results of the studies of the blood of the test animals were the same as those observed in the controls. Tables #E-1; E-1,a; E-1,b; E-1,c; E-1,d.

Experiment #71-B, Reproductivity and Second Generation Study

Procedure, Reproductivity Study

60 albino rats were selected in litter mate pairs at weaning, 30 males and 30 females. One-half of these animals, 15 males and 15 females, served as controls and were fed Purina Laboratory Chow ad libitum until they reached 100 days of age. These animals were given tap water to drink. The other half of this group of animals, 15 males and 15 females, served as test animals and were treated in the same manner as the controls, except that the tests were given 10% caramel solution ad libitum as their sole source of fluid during their growing period. Growth curves were obtained on both test and control groups.

When the animals reached 100 days of age they were mated and records were kept of numbers of litters born and number of pups in each litter. These records were kept for a 200 day period.

Results, Reproductivity Study

Growth curves of the breeder rats are given in figure #2 and show no appreciable difference between tests and controls.

Records of number and size of litters of these animals are given in table #F-1. These data also show no appreciable differences between test and control animals.

Procedure, Second Generation Study

25 males and 25 females, which were offspring of the test animals in Experiment #71-B, were selected at random at weaning age. These animals were fed Purina Laboratory Chow ad libitum and given 10% caramel solution as sole source of fluid until they reached 100 days of age. Records were kept of the growth of these animals and at the end of the 100 day period they were autopsied and sections from 4 animals fixed for histological examination.

Hemoglobin, red and white cell counts, and differential counts were made on the blood of 8 of these animals.

Results, Second Generation Study

Growth curves of the second generation animals, as may be seen in figure #3, were normal. Hemoglobin values and blood counts were also normal. Table #E-1; E-1,e.

Histological examination of the tissues of these animals revealed no abnormalities which could be construed as having been caused by the ingestion of caramel.

Experiment #72-x, Caramel #S-4251

Procedure

32 albino rats (40-55 gm.) were selected shortly after weaning; 16 were used as controls and their litter mates (16) were used as tests. All the animals were fed Purina Laboratory Chow ad libitum. The control animals were given water to drink while the tests were offered 30 ml. of a 10% solution of caramel #S-4251 to drink. Records were kept of growth and caramel intake of these animals for 100 days. At the end of this period the animals were autopsied, hemoglobin and complete blood counts made, and sections of 4 rats fixed for histological examination.

Results

As may be seen in figure #4 there are no appreciable differences in the growth curves of test and control animals.

Tables #E-2 and E-2,b give the results of the blood examination. Here also are found no appreciable differences in test and control groups.

Histological examination of the tissues of these animals failed to show any abnormalities which may be construed as having been caused by the ingestion of this caramel.

Experiment #72-y, Caramel #U-L-11143

Procedure

This experiment was an exact duplicate of Experiment #72-x except that caramel #U-L-11143 was used instead of caramel #S-4251.

Results

All negative as in Experiment #72-x. Figure #5. Tables #E-2 and #E-2,c.

Experiment #72-A

Procedure

5 rats, 40-55 gm. in weight, were given a 20% solution of caramel #S-4251 as sole source of fluid for 100 day. Another group of 5 rats were given a 20% solution of caramel #U-L-14143 for the same length of time.

Results

Growth curves were normal as shown in figure #6.

Blood pictures of these animals were also normal as shown in tables #E-2, E-2b, and E-2c.

The Absorption of Caramel by Albino Rats

In view of the observations on the degree of color and the consistency of the fecal output of the rats with a large intake of caramel, it was deemed advisable to see if an estimate of the amount of caramel absorbed by the rats could be obtained. The following experiment was done to attempt to answer this question.

Procedure

Part 1

Two control and four test rats which had been drinking approximately 30 ml. of 10% caramel solution daily for 100 days were placed in metabolism cages for a 48 hour period. Total caramel intake was recorded and the feces from these animals were collected. The procedure for the estimation of the amount of caramel absorbed by the test rats was as follows: The feces of the test rats were leached with hot water and the extract was made up to a standard volume. The feces of the control animals were also leached with hot water and made up to the same volume as the tests. A known amount of caramel was added to the extract from the feces of the controls. This solution was used as a standard for comparison with the solution obtained from the test rats. It was necessary to use the extract from the control feces in making up the standard caramel solution because allowance had to be made for the color in normal rat feces. The difference in the amount of caramel in the feces of each test rat and the amount of caramel ingested by each rat is reported as caramel absorbed.

This procedure was repeated on two of these rats drinking 10% caramel and also on one rat drinking 20% caramel.

Part 2

Three rats that had been drinking 10% caramel and two rats that had been drinking 20% caramel were given water to drink for a 24 hour period and then given caramel for 24 hours and then water for 24 hours. The fecal output was collected for the last 48 hours. After a few days this procedure was repeated using the same rats, and again several days later.

Part 3

Three rats were given a 10% solution made from dehydrated caramel; one rat drank the regular 10% caramel and one rat the regular 20% caramel. The same procedure was followed as in part 2.

Results

The results may be seen in table #D-1.

Conclusions

While there do occur rather wide variations in caramel absorption from day to day, the average per cent of the ingested caramel which was absorbed remained rather constant throughout the experiment. We may conclude from these few observations that approximately one-third of the color-giving components of the caramel may be absorbed by the albino rat. As concerns those components of the caramel which do not account for the color we can draw no conclusions.

2-12-51

The Utilization of Caramel by Albino Rats.

Part 1.

Two control rats which drank water and 4 test rats which had been drinking approximately 30 ml. 10% caramel solution daily for about 100 days were placed in metabolism cages for the collection of the fecal output. They were left in these cages for a 48 hour period, their caramel intake recorded and fecal output collected. The color was dissolved out of the feces with hot water. The color of the extract was compared with a caramel standard to determine the total caramel output of each rat. The difference between output in the feces and intake is reported as caramel which was utiliaed by the rats. This amount, of course is based on the amount of color which was lost. The fecal output of the controls was used as a blank.

Part 2.

Three rats which had been drinking 10% caramel and two rats which had been drinking 20% caramel were giveh water to drink for a 24 hr. period and then given caramel for 24 hours and then water for 24 hrs. The fecal output was collected for the last 48 hours- 24 of which the animals were on caramel. After a few days this procedure was repeated using the same rats.

Part 3.

Three rats were given a 10% solution made from dehydrated caramel; one rat drank the regular 10% caramel and one rat drank the regular 20% caramel. The same procedure being followed as in Part 2.

Data are on attached sheet.

Conclusions:

While there does occur rather wide variations in caramel utilizatcn from day to day, the average percent of the ingested caramel which is utilized remaind rather constant throughout the experiment. It seems to make no difference whether the amount of caramel is large or small that is ingested . We may conclude from these few observations that it seems that approximately one-third of the color giving components of the caramel may be utilized by the albino rat. As concerns those components of the carmel which do not account for the color we can draw no conclusions.

E. Simpson Wynn

Coconut utilization

	Coconut	WATER gms.	COMET gms	USED gms	USED % of WATER	
1	10% Solution	5.0	4.0	1.0	20	Part I
	"	6.0	4.2	1.8	30	48 hours
	"	2.9	2.1	0.8	28	
	"	3.2	1.8	1.4	44	avg. 31%
	10% Solution	2.7	2.0	0.7	26	Part II
	"	1.3	0.8	0.5	38	24 hours
	"	1.9	0.9	1.0	53	
	20% Solution	3.6	2.4	1.2	33	
	"	3.4	2.4	1.0	29	avg. 36%
	10% Solution	1.1	0.9	0.2	18	
	"	1.3	0.9	0.4	31	48 hours
	"	1.1	0.8	0.3	27	
	20% Solution	2.6	1.9	0.7	23	
	"	2.4	1.9	0.5	21	avg. 24%
	10% Solution (Dried)	2.5	1.6	0.9	36	Part III
	"	1.6	1.2	0.4	25	
	Reg. 10% Sol.	2.8	2.0	0.8	29	24 hours
	10% Solution (Dried)	3.0	2.5	0.5	17	
	Reg. 20% Sol.	6.0	2.6	3.4	57	avg. 33%

Grand avg. 31%

Histological Examination of Tissues

Procedure

At the end of each section of the experiments on the study of ingestion of caramel by albino rats, a representative group of animals were autopsied and slices taken and fixed (one in Zenker's fluid and one in 10% formalin) from each of the following organs: liver, spleen, kidney, stomach, heart, lungs, pancreas, gonads, duodenum, small intestine, and large intestine. After fixation these slices were sent to Dr. Walter Sheldon, pathologist at Grady hospital, for staining, sectioning and histological examination.

Dr. Sheldon's reports will be found attached to this report.

Results

Dr. Sheldon's reports all show that there was no damage to any of the organs of these animals which could be construed as having been caused by the ingestion of caramel.

Charles

WALTER H. SHELDON, M. D.
36 BUTLER STREET, S. E. CYpress 3096
ATLANTA 3, GEORGIA

February 20, 1951

Dr. John Haldi
Physiology Building
Emory University
Emory University, Georgia

Report on rat tissues submitted under your experimental accession Nos. 16, 20, 29 and 30.

Under Nos. 16 and 20 the following tissues were submitted: Heart, lung, spleen, pancreas, liver, stomach, various segments of intestinal tract, kidney, adrenal, testicle and thyroid. The sections were Zenker's fixed and were stained with phloxine-methylene blue. Upon histologic study no remarkable findings were encountered.

Under Nos. 29 and 30 the following tissues were submitted: Heart, lung, pancreas, liver, stomach, various segments of intestinal tract, kidney, adrenal, testicle and thyroid. The material was Zenker's fixed and the sections were stained with phloxine-methylene blue. Upon histologic study no remarkable findings were encountered.

Upon comparison of tissues from the different animals with each other no differences were noted.

WHSheldon
W. H. Sheldon, M. D.

WHS/fw

Nos. 16 & 20 were given 10% Coconut oil emulsion in drinking ad libitum - equ. to 3 cc's per day for 130 days. Average daily consumption 23 and 27 cc's respectively; total consumption 2990 and 3510 cc's respectively.

Nos. 29 & 30 were given 10% Cocoa oil emulsion in drinking ad libitum - equ. to 3 cc's per day for 130 days. Average daily consumption 21 and 23 cc's respectively. Total consumption 3600 and 3510 cc's respectively.

WALTER H. SHELDON, M.D.
36 Butler Street, S. E. Cypress 3096
Atlanta 3, Georgia

February 20, 1951

Dr. John Haldi
Physiology Building
Emory University
Emory University, Georgia

Report on rat tissues submitted under your experimental accession Nos. 16, 20, 29 and 30.

Under Nos. 16 and 20 the following tissues were submitted: Heart, lung, spleen, pancreas, liver, stomach, various segments of intestinal tract, kidney, adrenal, testicle and thyroid. The sections were Zenker's fixed and were stained with phloxine-methylene blue. Upon histologic study no remarkable findings were encountered.

Under Nos. 29 and 30 the following tissues were submitted: Heart, lung, pancreas, liver, stomach, various segments of intestinal tract, kidney, adrenal, testicle and thyroid. The material was Zenker's fixed and the sections were stained with phloxine-methylene blue. Upon histologic study no remarkable findings were encountered.

Upon comparison of tissues from the different animals with each other no differences were noted.

S/ W. H. Sheldon

W. H. Sheldon, M. D.

Rats Nos. 16 and 20 were given 10% caramel solution to drink ad libitum up to 30 cc. per day for 130 days. Average daily consumption 23 and 27 cc., respectively; total consumption 2990 and 3510 cc.

Rats Nos. 29 and 30 were given 20% caramel solution to drink ad libitum up to 30 cc. per day for 130 days. Average daily consumption 28 and 27 cc., respectively; total consumption 3640 and 3510 cc. respectively.

COPY

February 20, 1951

Dr. John Haldi
Physiology Building
Emory University
Emory University, Georgia

Report on rat tissues submitted under your accession Nos. 16, 20, 29, and 30.

Under Nos. 16 and 20 the following tissues were submitted: Heart, lung, spleen, pancreas, liver, stomach, various segments of intestinal tract, kidney, adrenal, testicle and thyroid. Upon histologic study no remarkable findings were encountered.

Under Nos. 29 and 30 the following tissues were submitted: Heart, lung, spleen, pancreas, liver, stomach, various segments of intestinal tract, kidney, adrenal, testicle and thyriod. Upon histologic study no remarkable findings were encountered.

Upon comparison of tissues from the different animals with each other no differences were noted.

W. H. Sheldon, M. D.

WHS/fw

COPY

Note: Animals were from Experiment #71-Preliminary. Nos. 16 and 20 were given 10% caramel solution. Nos. 29 and 30 were given 20% caramel solution.

Chron

WALTER H. SHELDON, M. D.
36 BUTLER STREET, S. E. CYPRESS 3096
ATLANTA 3, GEORGIA

March 27, 1951

Dr. John Haldi
Physiology Building
Emory University
Emory University, Georgia

Additional report on rat tissue submitted under your experimental accession Nos. 16, 20, 29 and 30.

No. 16, 3 sections of brain, 1 section of pituitary and 1 section of striated muscle and cartilage under label of thyroid but not containing any thyroid tissue. Histologic studies reveal nothing of note.

No. 20, 3 sections of brain, 1 section of pituitary and 1 section of thyroid. Upon histologic study no remarkable findings are encountered.

No. 29, 3 sections of brain, 1 section of striated muscle labeled thyroid but not containing any thyroid tissue. No pituitary is submitted. Upon histologic study no remarkable findings are encountered.

No. 30, 2 sections of brain, 1 section of thyroid, no pituitary submitted. Upon histologic study no remarkable findings are encountered.

Comparison of the tissues from different animals with each other reveal no appreciable differences.

WHSheldon
Walter H. Sheldon, M. D.

WHS/fw

March 27, 1951

Dr. John Haldi
Physiology Building
Emory University
Emory University, Georgia

Additional report on rat tissues submitted under your experimental accession Nos. 13, 20, 29 and 30.

No. 13, 3 sections of brain, 1 section of pituitary and 1 section of striated muscle and cartilage under label of thyroid but not containing any thyroid tissue. Histologic studies reveal nothing of note.

No. 20, 3 sections of brain, 1 section of pituitary and 1 section of thyroid. Upon histologic study no remarkable findings are encountered.

No. 29, 3 sections of brain, 1 section of striated muscle labeled thyroid but not containing any thyroid tissue. No pituitary is submitted. Upon histologic study no remarkable findings are encountered.

No. 30, 2 sections of brain, 1 section of thyroid, no pituitary submitted. Upon histologic study no remarkable findings are encountered.

Comparison of the tissues from different animals with each other reveal no appreciable differences.

Walter H. Sheldon, M. D.

WHS:fw

COPY

Note: Animals were from Experiment #71-Preliminary. Nos. 13 and 20 were given 10% caramel solution. Nos. 29 and 30 were given 20% caramel solution.

WALTER H. SHELDON, M. D.
36 BUTLER STREET, S. E. CYpress 3096
ATLANTA 3, GEORGIA

May 24, 1951

Dr. John Haldi
Physiology Building
Emory University
Emory University, Georgia

Report on rat tissue submitted under your experimental accession Nos. 41, 43, 45 and 47.

The following tissues are submitted on all four animals. Heart, lung, spleen, stomach, small and large intestines, pancreas and liver. Sections of ovary are submitted under the Nos. 41, 45 and 47. A section of testicle was submitted under No. 43.

All sections are stained with hematoxylin and eosin. No abnormal findings of any sort could be detected except as follows. The lung section of animal No. 41 showed slight subacute interstitial pneumonitis and some brown pigment laden large mononuclear cells are present in the peripheral alveoli. The kidneys from the same animal showed minimal chronic interstitial nephritis.

The lung section from animal No. 45 showed minimal recent hemorrhage into the alveolar spaces. The findings encountered in these two animals do not appear particularly significant since I have observed similar findings in many normal rats sacrificed as controls.

Walter H. Sheldon, M. D.

WHS/fw

Exp. 11
100 day Regular Enamel
Test Rats

May 24, 1951

Dr. John Haldi
Physiology Building
Emory University
Emory University, Georgia

Report on rat tissue submitted under yourexperimental Accession Nos. 41, 43, 45, and 47.

The following tissues are submitted on all four animals. Heart, lung, spleen, stomach, small and large intestines, pancreas, and liver. Sections of ovary are submitted under the Nos. 41, 45, and 47. A section of testicle was submitted under No. 43.

No abnormalfindings of any sort could be detected except as follows. The lung section of animal No. 41 showed slight subacute interstitial pneumonitis and some brown pigment laden large mononuclear cells are present in the peripheral alveoli. The kidneys from the same animal showed minimal chronic interstitial nephritis.

The lung section from animal No. 45 showed minimal recent hemmorrage into the alveolar spaces. The findings encountered in these two animals do not appear particularly significant since I have observed similar findings in many normal rats sacrificed as controls.

Walter H. Sheldon, M. D.

WHS/fw

COPY

Note: These animals were on Experiment #71-A. They were given 10% caramel solution for 100 days.

EMORY UNIVERSITY
36 BUTLER STREET, S. E.
ATLANTA 3, GEORGIA

SCHOOL OF MEDICINE

June 18, 1951

Dr. John Haldi
Physiology Building
Emory University
Emory University, Georgia

Dear John:

This is another report on rat tissues bearing your accession numbers 1, 2, 3 and 4. The tissue was submitted fixed in Zenker's and sections were stained with hematoxylin and phloxine. The following organs were represented: Under numbers 1 and 2, heart, lung, spleen, stomach, intestinal tract, pancreas, liver, kidney and testicle. Under numbers 3 and 4 the same organs were present with the following exceptions; no spleen was submitted under number 3 and instead of testicles, ovaries were present in both numbers 3 and 4.

The only positive findings consisted in areas of bronchopneumonia and interstitial pneumonitis encountered in the lungs of numbers 1, 3 and 4. This process appeared to be of several days standing.

The testicles and ovaries showed evidence of good functional activity. There were no other remarkable findings.

The above described finding of bronchopneumonia and interstitial pneumonitis, I have not infrequently encountered in otherwise perfectly normal untreated rats.

Sincerely yours,

WHSheldon

Walter H. Sheldon, M. D.

WHS/fw

211 C generation
100 days.

Carmel (regular)

June 18, 1951

Dr, John Haldi
Physiology Building
Emory University
Emory University, Georgia

Dear John:

This is another report on rat tissues bearing your accession Nos. 1, 2, 3, and 4. The following organs were represented: Under nos. 1 and 2, heart, lung, spleen, stomach, intestinal tract, pancreas, liver, kidney, and testicle. Under Nos. 3 and 4 the same organs were present with the following exceptions: no spleen was submitted under No. 3 and instead of testicles, ovaries were present in Nos. 3 and 4.

The only positive findings consisted in areas of broncopneumonia and interstitial pneumonitis encountered in the lungs of Nos. 1,3, and 4. This process appeared to be of several days standing.

The testicles and ovaries showed evidence of good functional activity. There were no other remarkable findings.

The above described finding of broncopneumonia and interstitial pneumonitis, I have not infrequently encountered in otherwise perfectly normal untreated rats.

Sincerely yours,

Walter H. Sheldon, M.D.

WHS/fw

COPY

Note: These animals were from Experiment #71-B, 2nd generation test animals.

WALTER H. SHELDON, M. D.
36 BUTLER STREET, S. E. CYPRESS 3096
ATLANTA 3, GEORGIA

July 17, 1951

Dr. John Haldi
Physiology Building
Emory University
Emory University, Georgia

Dear John:

Here is another report on rat tissues bearing your accession numbers 21, 23, and 25. The material was submitted fixed in Zenker's and the sections were stained with hematoxylin-phloxine. The following organs were represented: Under number 21 - heart, spleen, stomach, intestinal tract, pancreas, liver, kidney, and testicle. Under number 23 - heart, lung, spleen, stomach, intestinal tract, pancreas, liver, kidney and ovary. Under number 25 - lung, spleen, stomach, intestinal tract, pancreas, kidney and testicle.

No positive findings were encountered in the material submitted.

Sincerely yours,

Walter

Walter H. Sheldon, M. D.

WHS/mh

200 day regular carried

July 17, 1951

Dr, John Haldi
Physiology Building
Emory University
Emory University, Georgia

Dear John:

Here is another report on rat tissues bearing your accession Nos. 21, 23, and 25. The following organs were represented: Under No. 21, heart, spleen, stomach, intestinal tract, pancreas, liver, kidney, and testicle. Under No. 23, heart, lung, spleen, stomach, intestinal tract, pancreas, liver, kidney, and ovary. Under no. 25- lung, spleen, stomach, intestinal tract, pancreas, kidney, and testicle.

No positive findings were encountered in the material submitted.

Sincerely yours,

Walter H Sheldon, M.D.

WHS/mh

COPY

Note: These animals were from Experiment #71-A and were given 10% caramel solution for 200 days.

EMORY UNIVERSITY
36 BUTLER STREET, S. E.
ATLANTA 3, GEORGIA

SCHOOL OF MEDICINE

August 1, 1951

Dr. John Haldi
Physiology Building
Emory University
Emory University, Georgia

Dear John:

Rat tissues bearing your accession numbers 1,3,5 and 7 were submitted in Zenker's fluid and the sections were stained with hematoxylin-phloxine. The following organs were represented in each case: heart, lung, spleen, stomach, intestinal tract, pancreas, liver, kidney and ovary. Only under number 3, testicle was present instead of ovary.

Positive findings were areas of subacute bronchopneumonia in the lungs of numbers 1 and 5. The pneumonic process appeared to be of several days duration. The myocardium of numbers 1 and 5 showed minimal fragmentation of the muscle fibers.

The ovaries and the testicle revealed good functional activity.

Many thanks for sending me this material.

Sincerely yours,

Walter H. Sheldon, M. D.

WHS/mh

EY 72
(and) Y journal
Test

August 1, 1951

Dr. John Haldi
Physiology Building
Emory University
Emony University, Georgia

Dear John,

Rat tissues bearing your accession nos. 1, 3, 5, and 7, were submitted. The following organs were represented in each case: heart, lung, spleen, stomach, intestinal tract, pancreas, liver, kidney, and ovary. Only under no. 3 testicle was present instead of ovary.

Positive findings were areas of subacute broncopneumonia in the lungs of nos. land 5. The pneumonic process appeared to be of several days duration. The myocardium of nos.1 and 5 showed minimal fragmentation of the muscle fibers.

The ovaries and the testicle revealed good functional activity.

Many thanks for sending me this material.

Sincerely yours,

Walter H. Sheldon, M.D.

WHS/mh

Copy

Note: These animals were test animals from Experiment #72-x.

WALTER H. SHELDON, M. D.
36 BUTLER STREET, S. E. CYPRESS 3096
ATLANTA 3, GEORGIA

August 31, 1951

Dr. John Haldi
Physiology Building
Emory University
Emory University, Georgia

Dear John:

Rat tissues bearing your accession numbers 1, 3, 33, 35, 37 and 39 were submitted in Zenker's fixative. The sections were stained with hematoxylin-phloxine. The following organs were represented in each case: heart, lung, spleen, stomach, intestinal tract, pancreas, liver and kidney. Testicles were present in numbers 1, 3 and 35. Ovaries were present in numbers 33, 37 and 39.

Positive findings were foci of subacute and chronic bronchopneumonia in animals 1 and 35. The lungs in animals 3 and 33 showed minimal interstitial pneumonitis.

The testicles and ovaries revealed a normal functional pattern.

Many thanks for sending me this material.

Sincerely yours,

Walter

Walter H. Sheldon, M. D.

WHS/mh

33, 35, 37, 39
Exp't 72Y 100 days special caramel

#1 1, 3 -
Exp't 71 300 days regular caramel

August 31, 1951

Dr. John Baldi
Physiology Building
Emory University
Emory University, Georgia

Dear John,

Rat tissues bearing your accession nos. 1, 3, 33, 35, 37, and 39 were submitted. The following organs were represented in each case: heart, lung, spleen, stomach, intestinal tract, pancreas, liver and kidney. Testicles were present in nos. 1, 3, and 35. Ovaries were present in nos. 33, 37, and 39.

Positive findings were foci of subacute and chronic bronco-pneumonia in animals 1 and 35. The lungs in animals 3 and 33 showed minimal interstitial pneumonitis.

The testicles and ovaries revealed a normal functional pattern.

Many thanks for sending me this material.

Sincerely yours,

Walter H. Sheldon, M.D.

WHS/mh

COPY

Note; - Animals no. 1 and 3 were from Experiment #71-A and were given 10% caramel for 300 days. Rats nos. 33, 35, 37, and 39 were from Experiment #72-y.

EMORY UNIVERSITY
36 BUTLER STREET, S. E.
ATLANTA 3, GEORGIA

SCHOOL OF MEDICINE

October 3, 1951

Dr. John Haldi
Physiology Building
Emory University
Emory University, Georgia

Dear John:

Rat tissues bearing your accession numbers 15 and 17 were submitted in Zenker's fixative. The sections were stained with hematoxylin-phloxine. The following organs were represented in each case: heart, lung, spleen, stomach, intestinal tract, pancreas, liver and kidney. In addition a section of ovary was included under number 17.

The only significant finding consists in a few minute foci of lymphocytic infiltration in the kidney of number 17. I have encountered such findings in normal rats. The ovary revealed a normal pattern.

Thank you for sending me this material.

Sincerely yours,

Walter

Walter H. Sheldon, M. D.

WHS/mh

Ex 71-A
300 day

October 3, 1951

Dr. John Haldi
Physiology Building
Emory University
Emory University, Georgia

Dear John:

Rat tissues bearing your accession numbers 15 and 17 were submitted. The following organs were represented in each case: heart, lung, spleen, stomach, intestinal tract, pancreas, liver and kidney. In addition a section of ovary was included under number 17.

The only significant finding consists in a few minute foci of lymphocytic infiltration in the kidney of number 17. I have encountered such findings in normal rats. The ovary revealed a normal pattern.

Thank you for sending me this material.

Sincerely yours,

Walter H. Sheldon, M.D.

WHS/mh

COPY

Note: These animals were from Experiment 71-A and were given 10% caramel for 300 days.

Growth Record (Average Weight)

Test (10% Caramel) Control (H_2O)

Experiment 71-A a, b, c and d.

Days	0	10	20	30	40	50	60	70	80	90	100	110	120	130	140	150
Male - Test Table A-1,a	48	87	119	163	194	218	239	248	264	272	276	278	279	285	294	292
Male - Control Table A-1,b	47	91	129	168	199	219	234	254	264	273	280	285	284	288	288	291
Female - Test Table A-1,c	45	80	110	131	149	162	171	176	182	188	189	186	190	194	196	192
Female - Control Table A-1,d	45	76	105	129	146	159	165	169	175	179	181	183	188	187	187	188

Days	160	170	180	190	200	210	220	230	240	250	260	270	280	290	300
Male - Test Table A-1,a	292	300	306	312	312	316	321	321	319	321	324	318	329	321	323
Male - Control Table A-1,b	292	301	301	304	306	306	314	311	307	308	307	304	294	315	321
Female - Test Table A-1,c	200	194	201	201	199	207	211	216	216	213	215	214	219	215	216
Female - Control Table A-1,d	188	176	194	198	200	193	187	202	202	203	208	206	200	203	202

Table A-2, a and b.

Ex Ti-B

Growth Curves

	Test								Males
Days	0	10	20	30	40	50	60	70	80
Grams	48	77	106	138	172	203	221	238	252
	Control								Males
Grams	48	84	121	152	186	218	235	250	260

Table A-2,b

	Test								Females
Grams	45	73	97	124	144	159	165	174	180
	Control								Females
Grams	44	74	99	124	144	160	172	181	185

Table A-3, a and b.

2nd Generation (Caramel)

Average Weight

Ex. 71-3 Males

Days	0	10	20	30	40	50	60	70	80
Grams	35	62	101	142	171	202	223	237	246

Table A-3,b

Females

Grams	31	62	83	112	128	146	158	164	169

Growth Record

Experiment 72-X

Table A-4
Table A-6 below

	0	10	20	30	40	50	60	70	80	90	100
Control for Caramel X											
Males Average	43	80	128	175	206	230	254	260	268	277	283
Females Average	42	77	118	142	146	172	179	186	196	198	198
Test for Caramel X											
Males Average	43	83	124	157	202	229	247	253	274	282	286
Females Average	42	78	115	138	158	163	173	180	184	188	188
20% Caramel X										Table A-6	
Average	47	71	106	138	176	181	183	213	223	232	236

Growth Record

Experiment 72

Table A-5
Table A-6 y below

	0	10	20	30	40	50	60	70	80	90	100
					Control for Caromel Y						
Males Average	45	89	132	171	210	235	236	260	273	289	293
Females Average	39	74	106	129	143	159	165	173	183	186	188
					Test for Caromel						
Males Average	45	91	137	173	214	242	256	269	283	284	288
Females Average	39	75	112	136	154	164	177	181	190	194	194
20% Caromel Y										Table A-6	
Average	35	61	96	140	173	204	224	232	242	261	247

Daily Caramel Readings

Ex. 71 - Preliminary

Table B-1

10% Caramel				20% Caramel			
Rat No.	Total cc	No. days	Average cc per day	Rat No.	Total cc	No days	Average cc per day
16	2954	126	23	26	3339	127	26
17	3302	127	26	27	3494	131	27
18	3675	126	29	28	3283	126	26
19	3533	127	28	29	3601	127	28
20	3437	127	27	30	3436	127	27
Average	3380	127	27		3431	128	27

Total gms	Average gm. per day	Total gms	Average gms per day
338	2.7	686	5.4

Average
Daily Liquid Intake

Experiment 71-A

Table B-2

Males and Females (Combined)

	Total cc	No. Days	Average cc per day
Test	2238	100	22
	4603	197	24
	7551	289	26
Control	2363	103	23
	4285	193	22
	7187	284	25

Average
Daily Caramel Readings

Table B-3

	Total cc	No. Days	Average cc per day
Ex. 72-x	2011	95	21
Ex. 72-y	2008	93	22

Food Intake Record

Experiment 72-x

Table C-1

	10	20	30	40	50	60	70	80	90	100	Total Intake
						Test					
Males Average	84	130	159	156	165	166	150	160	149	154	1479
Females Average	85	127	125	136	170	153	149	144	156	163	1407
						Control					
Males Average	90	132	168	166	175	167	168	161	167	175	1568
Females Average	94	140	156	140	149	134	140	134	140	122	1348

Food Intake Record

Experiment 72-y

Table C-2

	10	20	30	40	50	60	70	80	90	100	Total Intake
						Test					
Males Average	111	147	154	173	176	158	187	174	192	186	1659
Females Average	98	133	133	135	110	130	148	151	152	157	1345
						Control					
Males Average	111	147	143	180	169	121	173	162	168	156	1557
Females Average	92	128	132	146	143	118	140	158	152	153	1349

Blood Averages (10% Regular Caramel and Controls)

Experiment 71-A — Table E-1

Condition	No. of Rats on Ex.	No. of Days on Ex.	RBC (Millions)	WBC (Thousands)	Hemoglobin gms. %
Males					
Test (10% Reg.)	9	100	8.8	16.5	18.0
Controls	8	100	8.1	16.7	17.8
Test (10% Reg.)	5	200	9.9	13.5	17.6
Control	3	200	9.0	11.1	17.5
Test (10% Reg.)	6	300	9.8	12.4	17.0
Control	7	300	9.9	13.7	16.6
2nd Gen. Test (10% Reg.)	4	100	9.1	19.1	18.5
Females					
Test (10% Reg.)	7	100	9.2	13.8	16.8
Control	8	100	8.6	13.7	17.3
Test (10% Reg.)	4	200	9.3	11.8	17.0
Control	3	200	8.9	9.5	17.1
Test (10% Reg.)	2	300	9.1	11.5	17.5
Control	2	300	9.0	10.4	15.5
2nd Gen. Test (10% Reg.)	4	200	9.7	14.7	17.2

Blood Averages (10% Regular Caramel and Controls)

Experiment 71-A

Table E-1

Lymphocytes			Neutrophils					
Small	Large	Combined	Segment	Band	Combined	EOS	Mono	Baso
73	7	80	10	4	14	5	1	0
69	6	75	17	4	21	5	1	0
70	5	75	15	8	23	2	0	0
70	5	75	17	8	25	3	0	0
	2	73	15	8	23	5	0	0
69	3	72	15	9	24	4	0	0
79	1	80	11	7	18	2	0	0
71	6	77	14	5	19	3	1	0
74	7	81	12	4	16	3	1	0
67	3	70	16	8	24	6	0	0
70	3	73	15	6	21	6	0	0
66	5	71	18	10	28	1	0	0
73	4	77	14	9	23	1	0	0
82	2	84	10	5	15	1	0	0

Blood Averages Caromels X-Y
10% and 20% + Controls

Experiment 72

Table E-2

Condition	No. of Rats on Ex.	No. of Days on Ex.	RBC (Millions)	WBC (Thousands)	Hemoglobin gms. %
Males					
Test (10% X)	5	100	9.7	11.2	17.1
Control	4	100	9.4	10.8	17.5
Test (10% Y)	8	100	9.9	13.2	18.1
Control	5	100	9.8	12.4	16.0
Test (20% X)	4	100	9.3	12.0	16.0
Test (20% Y)	4	100	9.5	9.7	17.1
Females					
Test (10% X)	6	100	9.9	10.9	17.4
Control	1	100	8.5	12.8	17.0
Text (10% Y)	8	100	9.8	10.1	16.7
Control	8	100	8.9	10.9	17.1

Blood Averages Caromels X-Y
10% and 20% + Controls

Experiment 72

Table E-2

Lymphocytes			Neutrophils					
Small	Large	Combined	Segment	Band	Combined	EOS	Mono	Baso
71	4	75	15	7	22	2	0	0
71	4	75	14	6	20	5	0	0
72	3	75	14	8	22	3	0	0
74	4	78	12	6	18	3	0	0
73	5	78	12	4	16	6	0	0
69	4	73	17	6	23	3	0	0
75	4	79	12	5	17	4	0	0
78	2	80	11	6	17	3	0	0
74	3	77	11	7	18	3	0	0
71	2	73	16	8	24	3	0	0

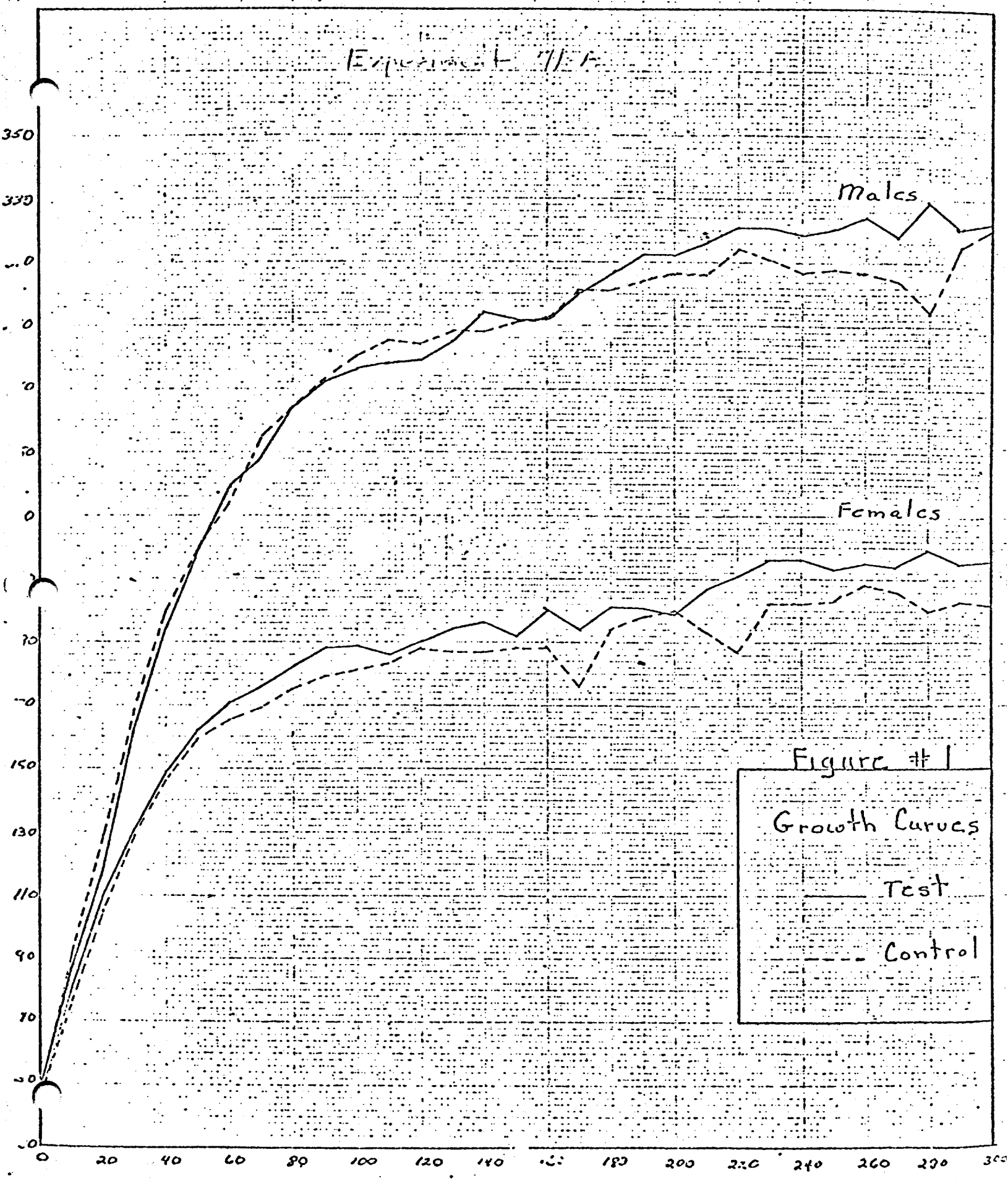
Table #F-1

Experiment #71-B

Reproductivity Record to Date

To date 16 pairs of animals, 8 test pairs and 8 control pairs, have been on the experiment for a period of 200 days. Given below are the totals of the litter records of these animals.

	Number of Breeders	Number of Litters Born	Number of Pups Born
Test	8	15	115
Control	8	16	123


Experiment "A"
Males
Females
Figure #1
Growth Curves
Test
Control
350
330
0
0
0
0
70
150
130
110
90
70
0
0
20
40
60
80
100
120
140
180
200
220
240
260
280
300
Days on Experiment

Experiment 71-B

Breeders

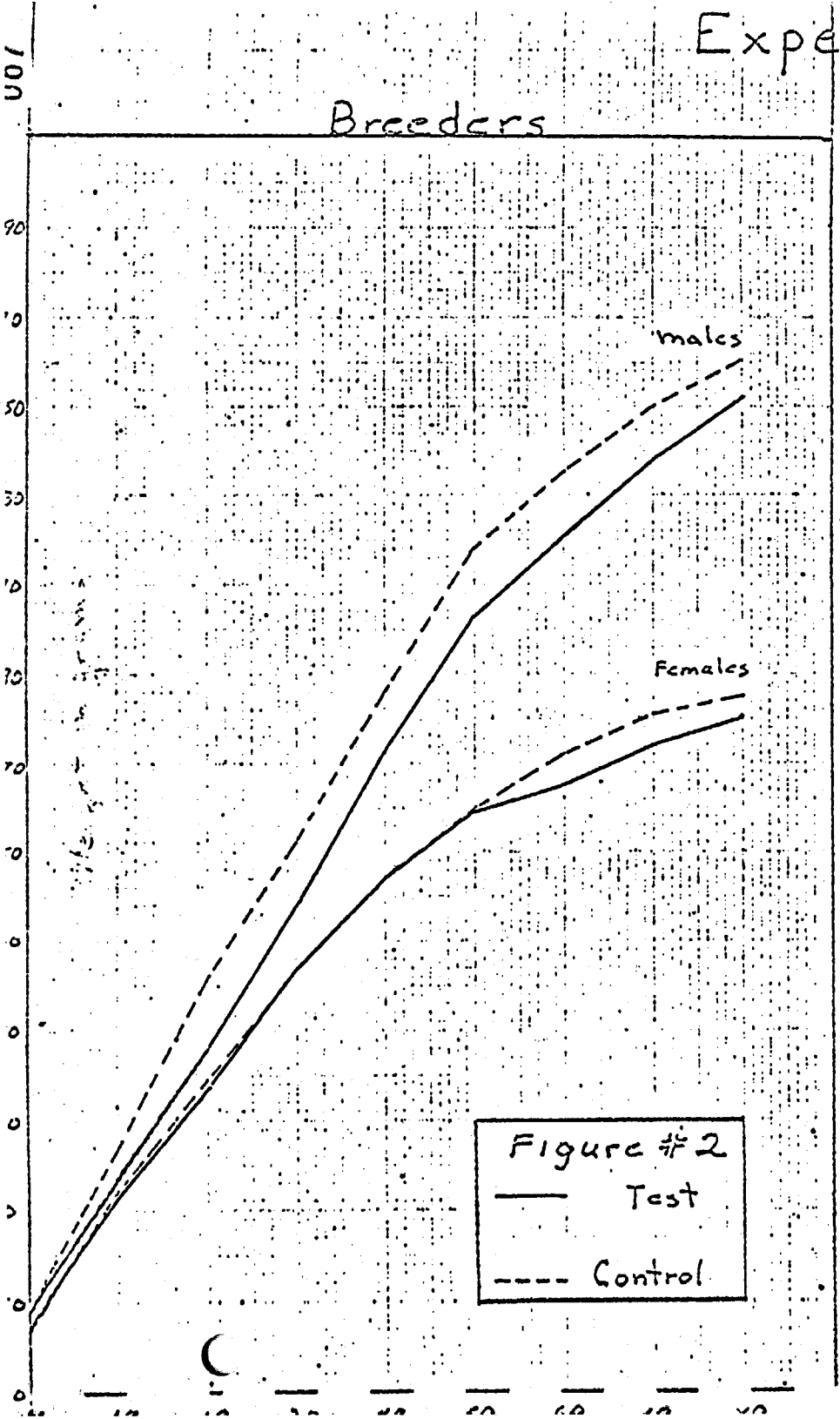


Figure #2

—— Test

---- Control

Second Generation

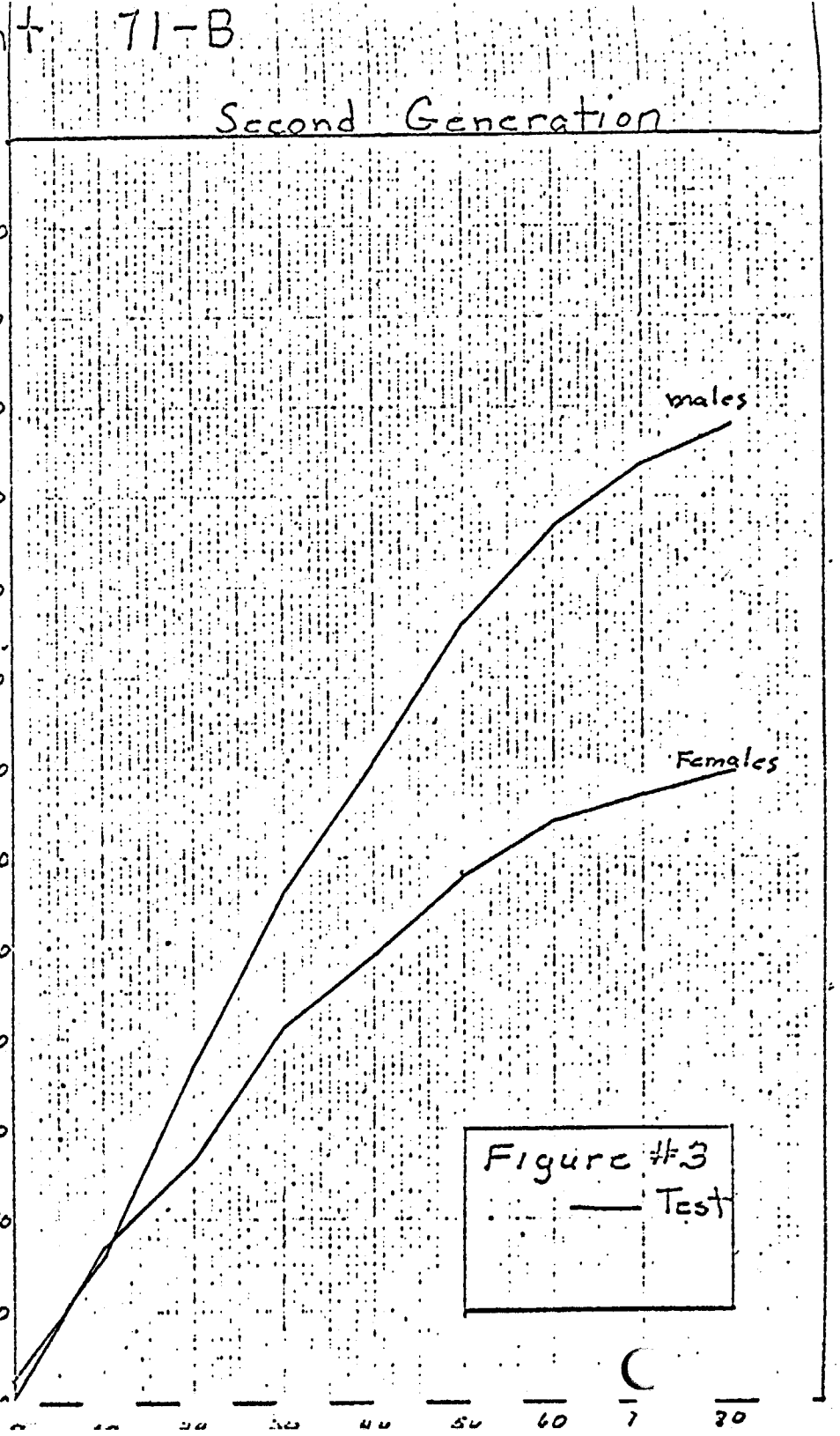
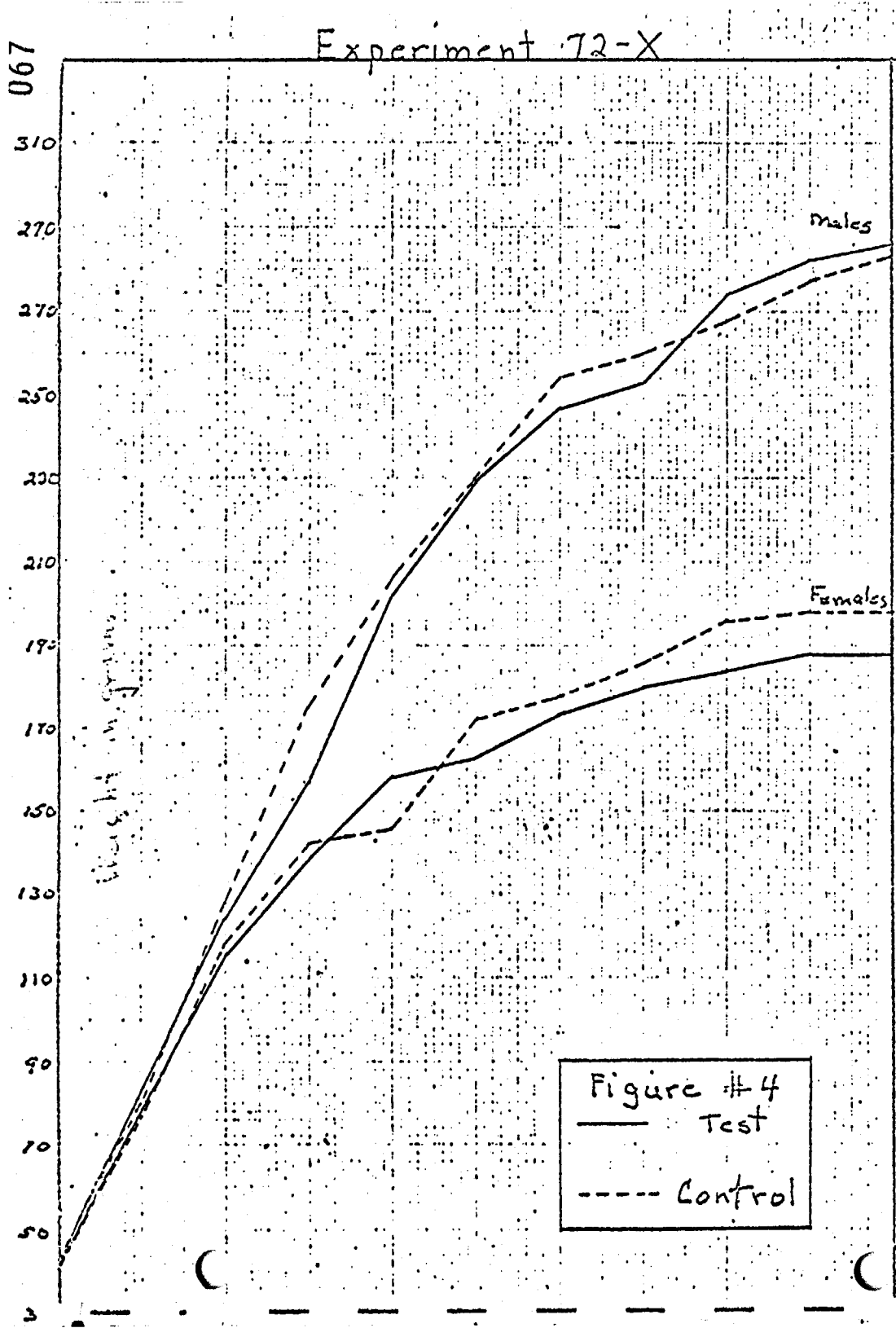
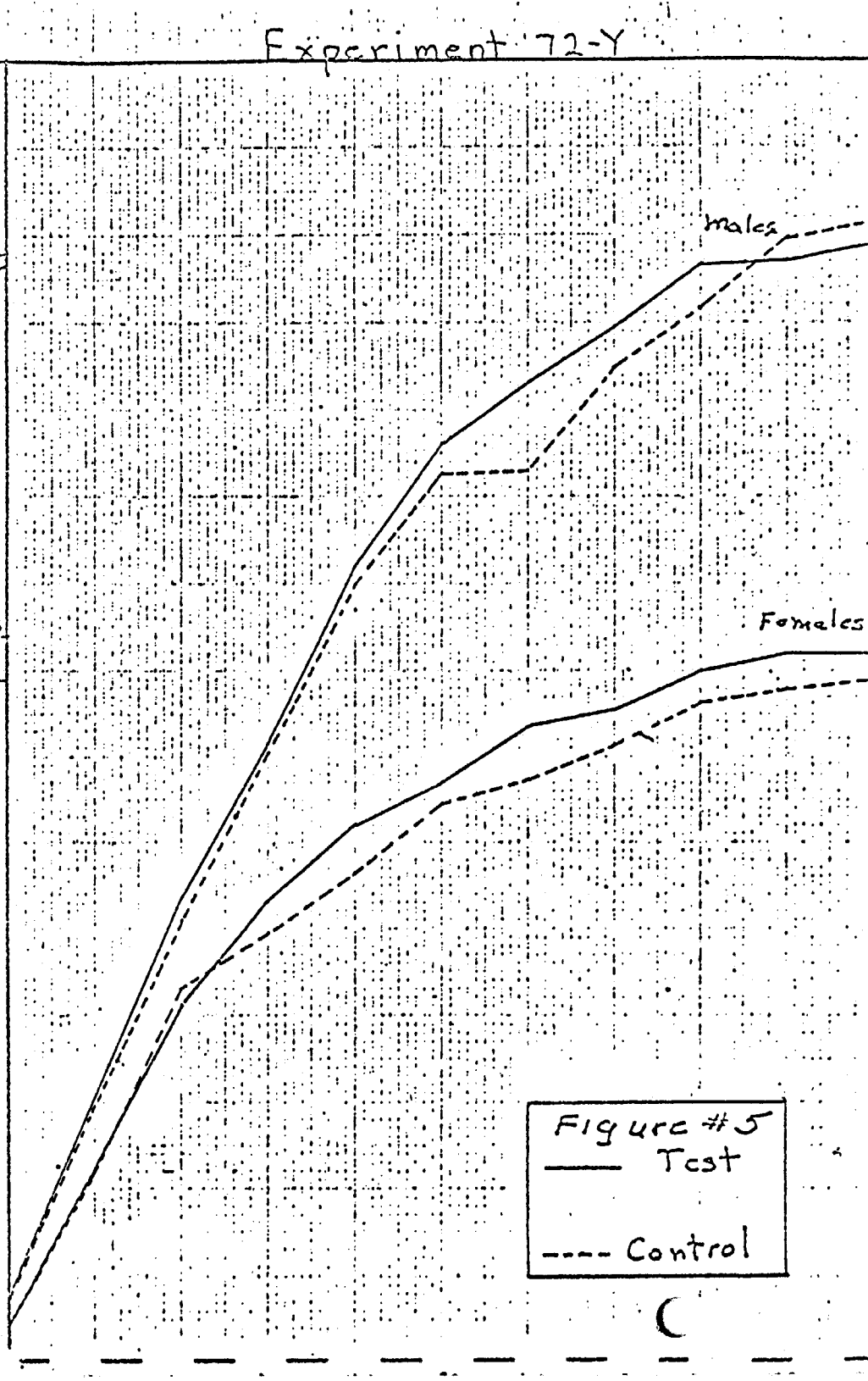


Figure #3

—— Test


Experiment 72-X
310
270
250
210
190
170
150
130
110
90
70
50
Males
Females
Figure #4
Test
Control


Experiment 72-Y
Males
Females
Figure #5
Test
Control

EXPERIMENT 72-A

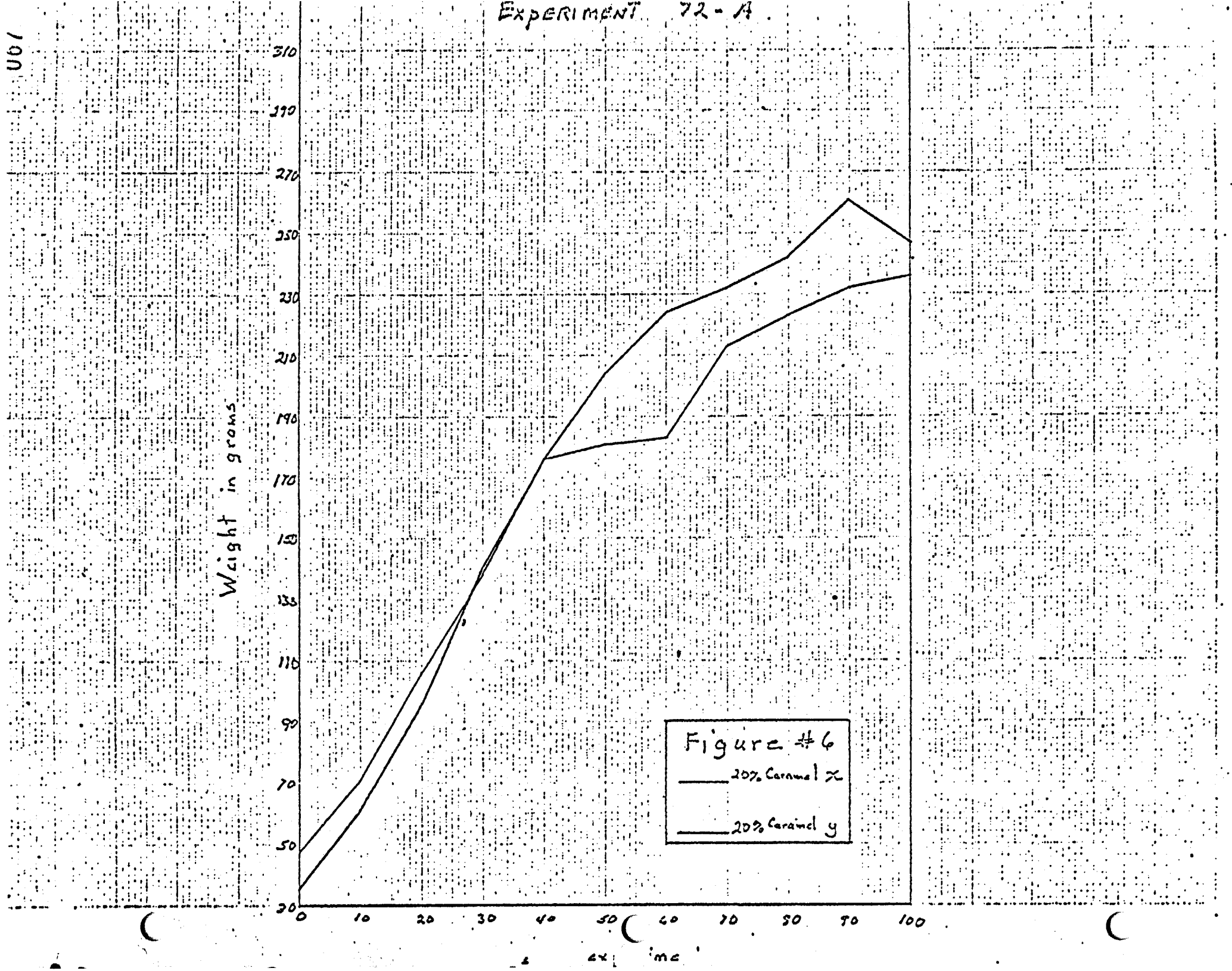


Figure #6

EXPERIMENT #73-X 1958

Purpose:-

This experiment was conducted to determine whether or not two caramels might be considered to be toxic when fed to rats. The caramels used were: 1) CPRCO, blend batch VE 1 and blend batch VE 2; 2) S-58, batch HX 79 and batch HX 80. One gallon of each of the 4 caramels were obtained from Mr. C. Gortatowsky.

Procedure:-

Twenty triplicate litter-mate groups of albino rats were placed on the experiment at weaning. The animals were allowed to eat ground Purina Laboratory Chow ad libitum and were given 1) water ad libitum; 2) 10% solution of CPRCO caramel and 3) 10% S-58 caramel. Each animal was offered 30 ml. of his respective fluid each day and his fluid intake was recorded.

The caramels were made up in the following manner: 150 gm. CPRCO, blend batch VE-1 and 150 gm. blend batch VE-2 were mixed and made up to 3 liters with distilled water; 150 gm. S-58 batch HX79 and 150 gm. batch HX80 were mixed and made up to 3 liters with distilled water to give a 10% solution. These solutions were stored under refrigeration and fresh solution was given to the animals each day.

The animals were weighed at ten-day intervals and their food intake was recorded. The experiment was conducted over a 100-day period at the end of which time the rats were sacrificed with an overdose of ether, gross pathological examination made and the following tissues were removed

for histological study: heart, lung, spleen, adrenal, kidney, pancreas, stomach, small and large intestine, liver and gonads. The tissues were fixed in neutral formalin.

Results:-

The growth curves of the control and test animals were almost identical. Net gain in weight, food intake and fluid intake are given in Table 1. It may be noted that the food intake per gram of body weight gained was slightly greater in the controls than in the tests. This probably can be explained by the fact that the caramels have a certain caloric value. It has been shown previously (see 1951 report on Caramel) that a portion of the ingested caramel is absorbed by the rat and this is very probably metabolized.

It also may be seen in Table 1 that the average intake of the caramels per kilo of body weight was greater in the females, 12.8 and 14.0 gms., than in the males, 11.2 and 11.5 gms. Using these values it may be calculated that at this level of intake a man of 75 kilos body weight would have ingested an average of over 800 grams of caramel per day and a woman of 60 kilos approximately the same amount each day.

There were no abnormalities noted on gross pathological examination in any of the 60 rats on the experiment. Histological examination of the tissues of 5 control animals, 5 tests on CPRCO caramel and 5 tests on S-58 caramel were negative

as may be seen in the attached report from Dr. Walter Sheldon.

Conclusions:-

The results of these studies indicate that these two caramels are not toxic when given to rats at an extremely high level.

Table 1

Net gain in weight, food intake and fluid intake of albino rats on Experiment #73-X

Fluid ingested	No. of animals	Sex	Avg. weight gain gms.	Avg. food intake gms.	Avg. fluid intake ml.	Avg. gm. #2/day per kilo of body weight
Water	7	M	261	16.5	26	--
CPRCO	7	M	271	15.3	25	11.2
S-58	7	M	260	15.6	24	11.5
Water	13	F	169	14.4	25	--
CPRCO	13	F	168	12.8	20	12.8
S-58	13	F	174	13.7	22	14.0

Department of Pathology

September 26, 1958

Dr. John Haldi
Department of Physiology
Emory University School of Dentistry
215 Basic Science Building
Atlanta 22, Georgia

Formalin fixed specimens bearing the identification exp. 73X, # 1-15 inclusive, were submitted and histologic sections were prepared. From each specimen sections representing heart, lung, spleen, pancreas, stomach, small and large intestines, liver, adrenal and kidney were examined. Numbers 1, 2, 3, 7, 8, 9, 10, 11 and 12 also included sections of ovary. Numbers 4, 5, 6, 13, 14 and 15 included sections of testicle. In some animals the following tissues were missing: #4 - liver and adrenal, #6 - adrenal, #8 - liver, #13 - adrenal, #15 - stomach and adrenal.

The histologic sections were stained with hematoxylin-phloxine and revealed no significant alterations. Virtually all lung sections showed occasional minute foci of interstitial pneumonitis, a finding which is also quite regularly encountered in normal animals.

Walter H. Sheldon, M.D.

WRS:ah

Rats # 1,4,7,10,13 CPRCO
2,5,8,11,14 Controls
3,6,9,12,15 S-58

cc: Dr. Glenville Giddings

CARAMEL COLORING

production composition functionality

By R. T. Linner
Director of Research
Sethness Products Co.
Chicago, Ill.

Caramel coloring is used in bakery products to impart a more colorful and attractive appearance. Because of this, it has been one of the basic ingredients in many bakery foods since it was first developed. Although caramel color has been available to the baker for many years, published information regarding the nature of the product and its many uses is scarce. The purpose of this article is to enlighten those who can and should be using caramel coloring in their bakery products. Caramel can impart many varying shades of color to a piece of bakery product, ranging from a light shade of tannish yellow all the way to a dark brown. It can produce eye-appealing, mouth-watering qualities in bakery foods such as breads, cakes, muffins, rolls, buns, icings, pies and pastries.

History

Caramel coloring was probably first made accidentally in the United States when the American Indians concentrated maple syrup or sap by dropping red hot stones into it. Caramel is obtained to some degree whenever sugars are heated alone, or when proteins and sugars are heated together. Natural progress, along with research and development, has led to improved raw materials, improved manufacturing plants, and much improved processes for producing caramel color on a commercial basis. Because of these facts, caramel for many years has been a well known, safe ingredient for use in the food industry.

Caramel coloring, sometimes called, "burnt sugar caramel," is described as "the amorphous, dark brown material resulting from the carefully controlled heat treatment of the foodgrade carbohydrates. Foodgrade acids, alkalis, and salts may be employed to assist caramelization in amounts consistent with good manufacturing practice." A more fully detailed description may be found in the "Federal Register" of June 25, 1963, Page 6498.

Raw Materials

In actual practice, caramel coloring is normally produced from liquid corn sugars or corn syrups, although cane sugar can be used. Only high quality sugars and syrups are used and each shipment is carefully checked for all properties known to affect the final caramel that is to be made. Some of the more important properties of the raw materials that must be checked, as well as their desired ranges, are listed in **Table I.**

Product Control

When research develops a formula and cooking procedure for making a particular type of caramel, the formula is proved by running many trial batches with many variations. Once the formula and cooking method have been proved, they are given to production. There are many types of caramel coloring and each type is specifically tailored to meet the needs of a specific use. Before, during, and after production takes over, the quality control laboratory analyzes the raw materials to make sure they are correct for proper caramelization. It then follows the production, making tests at intermediate stages, and finally making complete tests on the finished caramel before approving it for shipment. Caramel is shipped only after the testing has been completed and the caramel found to be within the required specification limits. Thus, research and development, and quality control give the laboratory a very important role in the caramel industry. The laboratory also assists with technical service problems and is always ready to give aid or advice to a customer.

Processing and Equipment

A modern caramel manufacturing plant fully utilizes the advantages of stainless steel equipment. The production cookers, filters, pipelines, pumps, and storage tanks are all of type 316 stainless steel to guarantee the purity of the final product. During the manufacturing process, caramel literally touches nothing except stainless steel.

Recording thermometers are used on each production cooker to make a

TABLE I

Typical Physical Properties of Sugars and Syrups Used to Make Caramel Coloring.

pH — 4.0 to 4.8
Baume — 42.0 to 46.0 at 60°F.
Specific Gravity — 1.478 to 1.4646 at 60°F.
Per cent of Equivalent Dextrose — 70 to 90%.
Per cent Protein — 1.0% or less.

Reprinted from the April, 1965 issue of BAKERS DIGEST

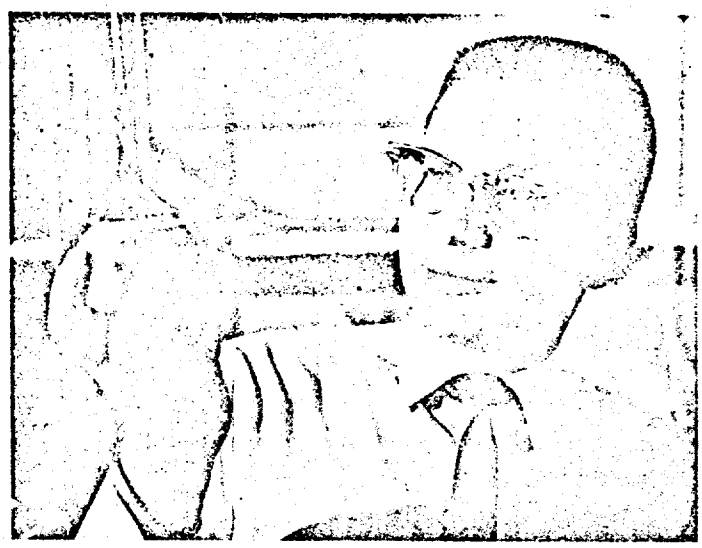
permanent record of the time-temperature relationship for each batch of caramel produced.

The liquid sugars or syrups are filled into clean autoclaves or cookers, and reactants are added according to the type of caramel to be produced. The size of the cookers varies greatly, but the smaller ones will produce quantities of caramel in the range of 8,000 to 10,000 pounds, while the larger ones can produce a batch up to 50,000 pounds. All cookers are equipped with stainless steel turbine type agitators or mixers. Continuous mixing is necessary to assure uniform heat transfer rates during the cook. The cookers are steam jacketed and the batch is held at a constant temperature above 250° F. for several hours, until the proper tinctorial strength is attained, when the batch of caramel coloring is quickly cooled to under 175° F. Caramel color is then pumped through stainless steel pressure-leaf filters, having a capacity of up to 40,000 pounds per hour. The caramel color is then pumped into a stainless steel storage tank, where it is held until the laboratory has checked it and the staff has standardized it for filling and shipping.

The cooking process is carried out and controlled with very great care, because too little coloring value is undesirable from the customer's standpoint, but too much cooking can "strain" the physical and chemical properties of the caramel. The viscosity and shelf life of a caramel can be reduced tremendously unless careful control is exercised at all times. Other desirable properties of the caramel can easily be destroyed if careful controls are not employed.

Important Properties

pH — The pH of baker's caramel is very important. pH shows the relative acidity of the caramel. It is defined as the reciprocal of the logarithm of the hydrogen ion concentration. pH may be illustrated as a series of consecutive numbers from 0 to 14, where 7 is neutral. As the value moves from 7 towards 0, the measure of the acidity is increased, and as the number moves from 7 toward 14, the measure of alkalinity increases. As pH is a logarithmic scale, a pH value of any given number represents ten times as much acidity as a pH of one whole number higher. In the opposite direction, any given pH represents ten times as much alkalinity as a pH of one whole number lower. Much care must be exercised in producing the correct pH value in the final caramel coloring. A water solution of a quality liquid baker's caramel should have a pH of approximately 4.0 plus or minus 0.7. This will fit it into the range of the normal fermentation pH of bakery goods.



R. T. Linner

Density or Weight Per Gallon — Baker's caramels may vary in density from 33.0 to 40.0 Baumé. The relationships between pounds per gallon and Baumé are given in **Table II.**

Color Strength — Even though the cost of any caramel coloring in a finished product is very small, the amount of actual coloring does vary quite considerably. Because of this, the color value or tinctorial strength is important to the user. Even so, it is not easy for the average user to determine the caramel color strength. The user therefore must either: (1) send the sample out to a testing laboratory; (2) buy equipment to make the tests himself; or (3) take the word of, and buy from, a reliable caramel manufacturer. Two common systems for testing color strength are used, namely the Klett and the Klett-Summerson instruments. They both use glass color standards to which a solution of caramel is matched. The Klett system uses a standard caramel glass. A one per cent solution of caramel is placed in a cup and is matched against the known glass standard. The Klett cup, containing the unknown caramel solution, is raised or lowered to present a longer or shorter path of light through the solution from the light source. The length of the light path is adjusted by raising or lowering the Klett cup to balance the color of the standard glass. The reading is made on a drum or wheel, and is called the Klett Color value or reading. The smaller the length of the light path, the greater the strength of the caramel, or as the Klett numbers decrease, the stronger the caramel. In the other system, the Klett-Summerson Colorimeter, a solution of caramel is placed in a special tube and the light transmitted through the solution is balanced against the light transmitted through replaceable standard glasses. Standard glasses of different maximum millimicron transmission can be used. A photocell picks up the light transmitted through the unknown caramel solution, and moves the indicator according to the color transmitted. A variable wheel then is adjusted by the operator to balance the light transmitted through the unknown caramel solution against the light transmitted through the standard glass used. When the balancing is finished, the Klett-Summerson color reading or value of the unknown is read off of the dial. With this instrument, the larger the number, the stronger the caramel solution.

TABLE II

BAUME	LBS. PER GALLON @ 60°F.
33.0	10.79
34.0	10.90
35.0	10.99
36.0	11.09
37.0	11.20
38.0	11.30
39.0	11.41
40.0	11.52

Caramel Spectrum — A very interesting sidelight on caramel coloring is that an analysis of its color spectrum shows its composition to be about 70 per cent red, 25 per cent yellow, and five per cent blue. If certified colors are used in trials to duplicate the color of caramel, it seems that about 60 per cent yellow, 35 per cent red, and five per cent blue are required. This difference seems to indicate that "caramel red" is much less intense than "certified red." Because of this, more yellow is needed to tone down the intensity of the red

into the yellow-orange range to make a synthetic color that will match caramel coloring.

On a spectrophotometer, the best wavelength at which to measure caramel coloring is between 560 and 650 millimicrons. In this range, particularly at 560 millimicrons, the best correlation and reproducibility are found. By calculation of wavelengths and percentages, though, the measurement should be made at a wavelength of 650 millimicrons. These facts merely indicate that caramel coloring does not form a true solution, and does not follow Beer's Law,* but instead is a colloidal solution.

Shelf Life – After the caramel is manufactured, it is stored in stainless steel tanks or packaged in specially developed plastic lined steel drums. The linings are approved food-grade linings. Caramelization, which ends in large molecular sizes polymers, continues after the manufacturing process is finished, although the reaction rate is very slow at room temperatures. After prolonged storage under adverse conditions, caramel color can resinify into an amorphous, irreversible gel, which is not usable. Since the reaction rate varies directly with the temperature, it is wise for the user to avoid storing caramel in an abnormally warm location. High quality caramel colors can be stored a year or longer at room temperature without adverse effects. A good policy to follow by the baker is to use his oldest stocks of caramel first, and not store caramel at temperatures above normal room temperatures.

Viscosity – The viscosity of caramel coloring is normally measured in terms of centipoises at 80° F. The Brookfield Viscosimeter is a convenient instrument for making these viscosity determinations. Viscosity is important to the baker, because low viscosity and high fluidity make it easier for the baker to handle and use caramel in his products.

Baker's caramels – Caramel is used to give a uniform shade that is darker than the baker could obtain in his goods economically without the use of small amounts of caramel coloring. Caramel for baking uses must have great coloring strength, must be free-flowing to be easily used, and generally should be tasteless in the final product unless the baker desires otherwise. Viscosity or thickness should not be confused with coloring strength. In most instances, free-flowing, low viscosity caramels are much stronger than the viscous grades. The fluid quality of caramel as used in the baking trade, therefore, is very important. This factor facilitates its solution in water and its equal dispersion in doughs. This is a must to guarantee a uniform shade in the finished product of breads, cookies, crackers, cakes, or other baked foods. The very slight flavor of caramel is due mainly to the natural "caramel" flavor derived from the raw material used, and to its natural slight acidity. But caramel imparts very little, if any, flavor to the bakery products in which it is used because the quantity used is normally quite small.

There are many grades of baker's caramel coloring. Some are manufactured to sell at a very low price, while some are manufactured for the buyer who discriminates on all of his raw materials. Obviously, cheap grades of caramel are not refined or processed as carefully as the better quality grades.

*An equation that relates light transmittance at given wavelengths to concentrations and length of light paths through the solutions.

Table III

Black Bread

Sponge	Lbs.	Ounces
Clear flour	9	—
Rye flour	45	—
Water	45	—
Yeast	1	14
Caramel	1	14
Emulsifier	—	12
Dough		
Clear flour	91	—
Water	45	—
Shortening	2	4
Salt	2	4
Corn Sugar	—	12
Molasses	—	12
Granulated caraway	1	8

Snack Rye

Sponge	Lbs.	Ounces
Clear flour	50	—
Water	30	—
Yeast	—	14
Yeast food	—	2
Caramel	—	12
Dough		
Clear flour	20	—
Rye flour	30	—
Water	36	—
Salt	2	—
Shortening	—	10
Sugar	—	6
Rye saver	1	12
Caraway seed	6	8
Pretzel salt	6	8
Mold inhibitor	1	—

Soft Dark Rye Bun

	Lbs.	Ounces
Soft Roll Dough	100	—
Caramel	5	2
Rye saver	2	9
Ground caraway	2	9

Devil's Food Cake

To make 100 one-pound cakes.

	Lbs.	Ounces
Cake flour	16	—
Shortening, emulsifier type	11	—
Salt	—	12
Sugar	22	—
Molasses	4	—
Caramel	—	12
Cocoa	3	2
Cottonseed color	3	4
Baking soda	—	3
Baking powder	—	12
Water	11	—
Dry Milk	2	6
then add		
Water	8	—
Whole Egg	9	8
Egg White	6	6
Vanilla	—	4

Basic Types of Caramels

A select grade of caramel is one that is tailor-made for the baking trade. It has a color strength value of 11.6 to 12.1 on the Klett Colorimeter, read as a one per cent solution. It has a pH of 3.6, plus or minus 0.3, a Baumé of 35.0, and a viscosity of 300 cps, plus or minus 100 cps at 80° F. It is approximately 65 per cent solids.

Caramel coloring can impart many varying shades of color to a piece of bakery product, from a light shade of tannish yellow all the way to a very dark brown. Caramel coloring can be used in any bakery product where a certain shade of color within the caramel spectrum is desired in the finished goods. One of the reasons caramel is so widely used in the baking field is because it can create such a wide range of colors. Caramel coloring, unlike certified color, adds measurable bulk to the mix. Since caramel coloring contains about 65 per cent solids, when the baker uses 10 pounds of caramel in his bread dough mix, he is adding six and one-half pounds to his total yield. Thus, the cost of using liquid caramel coloring is almost self-liquidating, as the cost of the caramel is returned to the baker when he sells his product.

Since liquid caramel contains approximately 35 per cent moisture, it may be troublesome to the baker in some mixes where he cannot tolerate this much moisture. The baker can eliminate moisture from the

caramel we have been discussing, but the moisture has been removed by drying. Only about half as much powdered caramel is required in a mix to give the same shade. Although the use of powdered caramel has many obvious advantages, the main disadvantage is a considerably higher cost. The baker will have to decide on the type of caramel color to use according to his own needs or particular problems. Powdered baker's caramel has a color strength of 5.5 to 6.5 on a Klett Colorimeter when read as a one per cent solution.

Basic Product Formulae

The formulae in **Table III** are not offered necessarily as good formulae, but are given merely to give some indication of the quantity and manner in which caramel is used in colored bakery products. The amount of color desired in any given product is a matter of personal taste, so it is recommended that the baker experiment to determine the exact color level he wants to achieve in a given product.

The following are some suggestions regarding the usage of caramel coloring in bread. Again, we would caution that the baker determine his own level of usage for caramel color to obtain the shade he wants.

(1) In dark type diet breads, use caramel up to one per cent or slightly higher for additional or for more uniform color.

(2) In the so-called black varieties of bread, use up to five per cent caramel, depending upon the color desired.

In straight dough, the caramel is added with the other ingredients. In the sponge-dough method, the caramel may be added to either the sponge or the dough after the water has been added. Actually, it may be easier to add it on the sponge side where the shortening is not involved.

Packaging

Caramel coloring is packaged in the following size containers: five gallon steel pails; 30 gallon steel drums; and 56 gallon steel drums. This complete line of steel drums is equipped with specially designed sanitary linings for the protection of the product. Modern rail tank-cars and tank trucks are also available for larger shipments.

THE END

Caramel

Caramel is a concentrated solution of the product obtained by heating sugar or glucose until the sweet taste is destroyed and a uniform dark brown mass results, a small amount of alkali, alkaline carbonate, or a trace of mineral acid being added while heating.

Description—Caramel is a thick, dark brown liquid with the characteristic odor of burnt sugar, and a pleasant, bitter taste. One part of Caramel dissolved in 1000 parts of water yields a clear solution having a distinct yellowish orange color. The color of this solution is not changed and no precipitate is formed after exposure to sunlight for 6 hours. Caramel spread in a thin layer on a glass plate appears homogeneous, reddish brown, and transparent.

Solubility—Caramel is miscible with water and is soluble in dilute alcohol up to 55 percent (v/v). It is immiscible with ether, chloroform, acetone, benzene, and solvent hexane.

Specific gravity, page 866—The specific gravity of Caramel is not less than 1.30.

Purity—The addition of 0.5 ml. of phosphoric acid to 20 ml. of a solution of Caramel (1 in 20) produces no precipitate.

Ash—Caramel swells when incinerated, and forms a coke-like charcoal which burns off only after prolonged heating at a high temperature. It yields not more than 8 percent of ash.

Packaging and storage—Preserve Caramel in tight containers.

CATEGORY—Color.

TOXICOLOGY AND APPLIED PHARMACOLOGY 14, 301–307 (1969)

Toxicity of Methylimidazoles

K. NISHIE, A. C. WAISS, JR., AND A. C. KEYL

Western Regional Research Laboratory, Agricultural Research Service, U.S. Department of Agriculture, Albany, California 94710[1]

Received June 26, 1968

Toxicity of Methylimidazoles. NISHIE, K., WAISS, A. C., JR., and KEYL, A. C. (1969). *Toxicol. Appl. Pharmacol.* **14**, 301–307. Toxic manifestations of ammoniated invert molasses in cattle can be simulated in laboratory animals such as rabbits, mice and day-old chicks by administering 4-methylimidazole (4-me-I). This compound, which results from the interaction of reducing sugars with ammonia, has previously been isolated from ammoniated invert molasses in addition to other compounds such as pyrazine derivatives. The mouse seems to be the most practical animal for testing the neurological signs produced by 4-me-I. The convulsive death produced by 4-me-I in the rabbit is characterized by the typical epileptiform EEG (high voltage, high frequency) concomitant with clonic and tonic seizures followed by tonic extensor seizure (high voltage, low frequency peaks) and terminal respiratory paralysis. This fatal course can be prevented by pentobarbital sodium and chlordiazepoxide. Diphenylhydantoin did not protect mice against 4-me-I seizures and death in contrast to phenobarbital sodium and chlordiazepoxide. 4-Methylimidazole had approximately one-fourth the convulsant potency of pentylenetetrazole. Imidazole, 1-methylimidazole, and 2-methylimidazole produced neurologic effects in mice similar to those caused by 4-me-I, but they were less potent as convulsants. The spontaneous motor activity of mice treated with 4-me-I was reduced to about 50% during the period preceding the seizures. In rabbits, 4-me-I decreased heart rate as much as 33% below the control and increased the respiratory rate to three times the control value during the period preceding the convulsions.

the past, the ammoniation of carbohydrate-containing materials such as molasses and citrus pulp was used to produce inexpensive nitrogen feed for cattle (Millar, Stiles, 1952; Wiggins and Wise, 1955; Wiggins, 1958), and it appeared to be a using source of nitrogen for animals (Knodt *et al.*, 1950, 1951; Magruder *et al.*, 1953; Magruder, 1954; Millar, 1941). However, violent "hysteria" was induced me animals receiving this feed (Wiggins, 1956a; Bartlett and Broster, 1958; ggins (1955, 1956a,b) and Wiggins and Wise (1955) found 10% imidazole and 20% zine derivatives in the ammoniated molasses. More recently, the list of potentially feeds was extended by the use of ammonia in the detoxification of castor bean

[1] Reference to a company or product name does not imply approval or recommendation of the product by the U.S. Department of Agriculture to the exclusion of others that may be suitable.

pomace and for the detoxification of mycotoxin-contaminated oil seed meals. In vie of the current interest in the safety of ammoniated commodities, it was decided to open the investigation on the toxicity of some of the compounds formed by interacti of ammonia with reducing sugars. The objective is to characterize the neurologi signs in order to provide a rationale for treatment and to find biological assays for detection of similar materials of unknown composition which might result from ammoniation of diverse agricultural commodities.

METHODS

Male albino mice weighing 20–25 g were used to determine: (a) the median con sant dose (CD50) and the median lethal dose (LD50) of the imidazoles, (b) the me protective dose (PD50) of anticonvulsants against the 90% convulsant dose methylimidazole (4-me-I), (c) the effects of 4-me-I on spontaneous motor act (SMA). A Woodard animal activity cage with 6 photocells and circular raceway used to measure SMA. Cumulative counts were recorded for individual mice (10 m dose) at 1-, 5-, 10-, and 15-min intervals after either saline (control) or 4-me-I had b administered ip. Values for CD50, LD50, and PD50 were determined using the i probit method of Miller and Tainter (1944) using 10 mice per dose and at least 30 m for each determination. The effects of 4-me-I on electroencephalogram (EEG) we determined in rabbits weighing 2.3–3 kg, employing chronic screw electrodes i planted in the skull. The electrodes were made of threaded nylon screws (1 8 in diameter, 3 4 to 1 4 inch long) with a stainless steel pin (1 32 inch) inserted in t center and protruding from the head of the screw. These electrodes were connect to a Beckman Type R, Dynograph by means of flexible standard EEG wire to whic silver coil was soldered. This silver coil had an inside diameter of 1 32 inch and fitt snugly over the pin protruding from the nylon screw electrodes. For EEG electro implantation, rabbits were anesthetized with pentobarbital sodium administered The distance between electrodes was determined by using one-half of the distan between the transverse sutures of the skull and 7–10 mm from the midline. Six ho 1 8 inch in diameter and 2.5–3 mm deep were drilled for the 3 pairs of EEG electroc (*F, P, O*: see Fig. 1). The wound healed in about 1 week, and the rabbits were th ready for use. One channel of the EEG was integrated for quantitation of EEG outp before and after the administration of 4-me-I. The effect of 4-me-I on respiration w recorded by placing a rubber pneumotachograph around the thoracoabdomi region of the rabbit and attaching it to a pressure transducer connected to the Bec man Dynograph recorder. The electrocardiogram (ECG) was recorded by locati one ECG electrode on the right shoulder and the other on the right hind quarter of t rabbit after shaving and cleaning these areas. The EEG, ECG, and respiration we recorded simultaneously. The anticonvulsant effects of chlordiazepoxide and pen barbital sodium were tested by iv injection at the onset of 4-me-I seizures in the rabb as evidenced by restlessness, hyperpnea, and twitching of the ears.

The CD50 and LD50 of 4-me-I were also determined in 1-day-old chicks (ma using a total of 60 chicks for both ip and po routes of administration. All compou used in this work were purchased from commercial sources with the exception 4-me-I, which was synthesized.

RESULTS

nvulsant Action of Imidazole and Methylimidazoles

Imidazole, 1-methylimidazole, 2-methylimidazole, and 4-methylimidazole (4-me-I) oduced convulsions in mice. The 4-me-I (found in the toxic ammoniated molasses) s the most potent convulsant of the imidazoles tested, although it was only one-irth as effective as the standard convulsant, pentylenetetrazole (Table 1). At high

TABLE 1

NVULSANT AND LETHAL EFFECTS OF IMIDAZOLES, PENTYLENETETRAZOLE, AND PYRAZINE IN MICE

Compound	pH, 15 mg ml in H_2O	CD50 ± SE (mg kg)		LD50 ± SE (mg kg)	
		ip	po	ip	po
ntylenetetrazole	7.8	38.5 ± 3.4	75 ± 5.2	134 ± 7.4	214 ± 15.5
Methylimidazole	8.2	155 ± 5	360 ± 18	165 ± 3	370 ± 15
(4-me-I)					
Methylimidazole	9.2	380 ± 8.2	1400 ± 79	380 ± 8.2	1400 ± 79
Methylimidazole	10.2	500 ± 12	1300 ± 70	480 ± 18	1400 ± 114
idazole	9.8	560 ± 34	1880 ± 45	610 ± 7.4	1880 ± 45
razine	6.8	Not convulsant		> 2000	> 2000

TABLE 2

ANTICONVULSANTS AGAINST 4-METHYLIMIDAZOLE[a] SEIZURES IN MICE

Anticonvulsants (doses tested, mg/kg)	PD50 ± SE, ip (mg/kg)	Pretreatment time of anticonvulsants (min)	Number of animals used
lordiazepoxide (3, 5, 7)	4.7 ± 0.4	20	30
enobarbital sodium (5, 6, 8, 10)	7 ± 0.5	60	40
phenylhydantoin sodium	inactive at 50 mg/kg	120	10

mg/kg ip (CD90).

TABLE 3

EFFECTS OF 4-METHYLIMIDAZOLE ON SPONTANEOUS MOTOR ACTIVITY OF MICE

Dose, ip	Cumulative counts ± SE			
	1 min	5 min	10 min	15 min
control	67 ± 17	241 ± 68	409 ± 117	554 ± 152
kg 4-me-I	53 ± 29	158 ± 74	193 ± 78	225 ± 152
g kg 4-me-I	49 ± 29	138 ± 81	176 ± 93	201 ± 93
g kg 4-me-I	30 ± 17	93 ± 31	151 ± 44	154 ± 52[a]

[a] 7/10 convulsed, 6/10 died.

dosage levels, all of the imidazoles tested produced varying degrees of tremor, restlessness, running, salorrhea, opisthotonus, Straub tail, and tonic extensor seizure terminating in death, whereas at lower doses loss of balance was the common finding. If pH of aqueous solutions of the convulsant methylimidazoles and pentylenetetrazole plotted against their respective CD50 values, an inverse linear relationship is obtained, i.e., the higher pH values corresponded to lower values of CD50 (Table 1). Convulsions were also produced in rabbits and day-old chicks (Tables 4 and 5). Base

TABLE 4

ANTICONVULSANTS AGAINST 4-METHYLIMIDAZOLE SEIZURES IN RABBITS

4-me-I (ip) (mg/kg)	Anticonvulsants[a]	Convulsion and death	Time of death (min)
50 (LD)	0	0/2	—
125	0	3/4	264–325
136	0	1/2	Overnight
150	0	3/3	18–100
150	iv 10 mg/kg pentobarbital sodium	0/3	—
150	iv 20 mg/kg pentobarbital sodium	0/1	—
150	iv 30 mg/kg pentobarbital sodium	0/1	—
150	iv 30 mg/kg chlordiazepoxide	0/4	—

[a] Anticonvulsants administered after seizures started.

TABLE 5

TOXICITY OF 4-METHYLIMIDAZOLE IN DAY-OLD LEGHORN CHICKS

Route of administration	CD50 ± SE (mg/kg)	LD50 ± SE (mg/kg)
ip	174 ± 10	210 ± 15
po	550 ± 30	590 ± 50

CD50 values, the decreasing order of sensitivity to 4-me-I for the 3 species is: rabbit > mouse ≥ chick. Toxic manifestations in the rabbit were similar to those appearing in mice with tonic extensor seizure characterized by abrupt paralysis of respiration, cyanosis, and gradual cardiac arrest (Fig. 1). In the rabbit, just prior to the convulsive seizure, the heart rate decreased in the order of 33%, below control, while the respiratory rate increased to three times normal values.

In the day-old chicks, doses of 100 mg/kg ip of 4-me-I caused tremors, peeping, and spreading of the wings; doses greater than 150 mg/kg ip caused, in addition, opisthotonus, running against the cage wall with spread wings, prostration with clonic leg movements, and terminal tonic extensor seizures. Chicks surviving the convulsive seizure lay prone on the cage floor unable to regain footing. Oral doses greater than 550 mg/kg produced diarrhea in addition to the convulsions, and although the onset of convulsions was dose related, there was much greater variation than seen when the compound was administered by ip route.

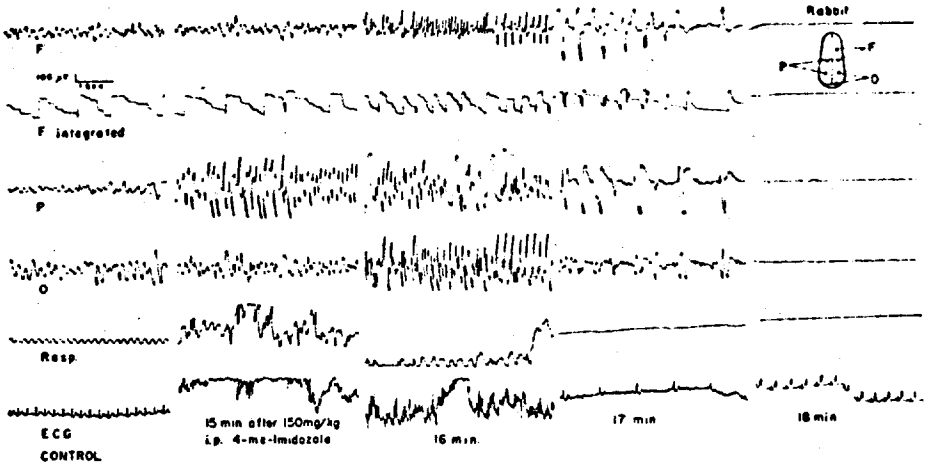


FIG. 1. Convulsive and lethal effect of ip 150 mg/kg 4-me-I in rabbit.

Effects of Anticonvulsants against 4-Methylimidazole Seizures

In mice, chlordiazepoxide and phenobarbital were effective in preventing 4-me-I seizures, while diphenylhydantoin at 50 mg/kg ip was ineffective (Table 2). In rabbits, chlordiazepoxide and pentobarbital sodium given iv at the onset of convulsions proceed by a lethal dose of 4-me-I prevented the epileptiform seizures and death (Fig. 2). In addition, the lowered cardiac rate returned to normal. Pentobarbital sodium abolished the hyperpnea caused by 4-me-I. If high doses (20–30 mg/kg iv) of pentobarbital were administered to convulsing rabbits, nystagmus and head drop were produced.

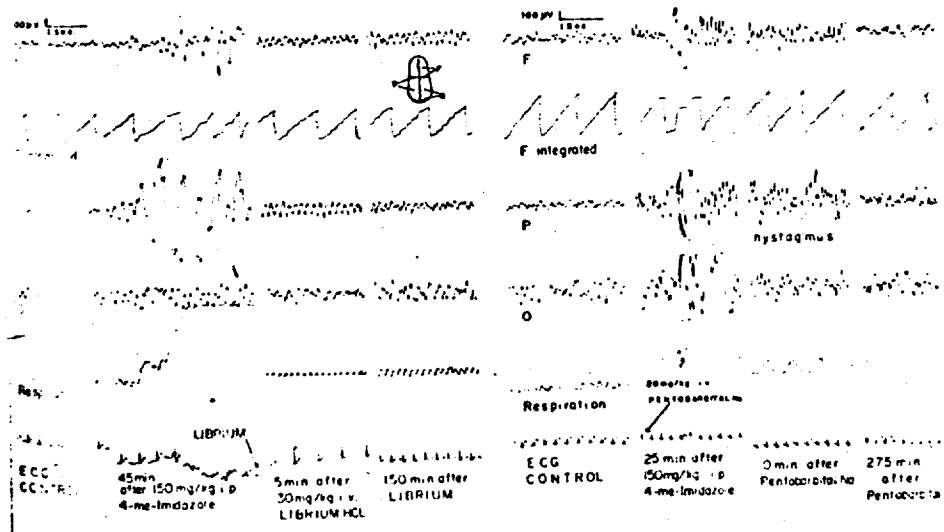


FIG. 2. Protective effect of chlordiazepoxide (Librium) and pentobarbital sodium against convulsive seizure and death caused by ip 150 mg/kg 4-me-I in the rabbit.

The lowest effective doses of anticonvulsants tested against convulsant dose of 4-me- (150 mg kg) in rabbits were 10 mg kg of pentobarbital sodium iv and 30 mg kg i chlordiazepoxide, the latter produced neither nystagmus nor head drop.

Effects of 4-Methylimidazole on Spontaneous Motor Activity in the Mouse

Subconvulsant (50-100 mg/kg ip) and convulsant (150 mg/kg ip) doses of 4- decreased spontaneous motor activity in mice to about 50% of the control acti- 15 min (Table 3), which corresponds to the preconvulsive period.

DISCUSSION

The compound 4-me-I, synthesized in this laboratory, produced neurologica in rabbits, mice, and chickens analogous to those described by Wiggins (1956 Bartlett and Broster (1958) in cattle and chicks fed with ammoniated invert m or its ethyl acetate extract. These authors described such signs as violent "hyst restlessness, bellowing, frothing at the mouth, paralysis of cattle, and madn chickens. The ammoniated molasses residue following ethyl acetate extraction. not toxic in contrast to its extract. Low molecular weight pyrazine and imida derivatives were identified in the extract by Wiggins (1956a) and were suspected as t toxic factors. However, he did not test these derivatives separately for toxicity. Th neurological similarities between signs described in this work in various laborato animals subjected to 4-me-I and those evoked in cattle fed with ammoniated produc suggest very strongly that 4-me-I is partly responsible for the toxicity, since it was o of the compounds found in the toxic extract. Our toxicologic findings on 4-m confirmed previous work (Leiter, 1925; Loeper *et al.*, 1935; Supniewski, 1928, Yam moto, 1941) on this compound. Pyrazine-derivatives were not tested due to their u availability and cannot be excluded. Pyrazine itself was neither toxic nor lethal at a g kg dose in mice.

Pentobarbital sodium and chlordiazepoxide prevented convulsive seizures in mi evoked by 4-me-I, whereas diphenylhydantoin increased the clonic leg movements mice and did not protect them against death. In this respect, the 4-me-I seizures r semble those produced by pentylenetetrazole, in which diphenylhydantoin may i crease clonic seizures (Goodman *et al.*, 1953). In rabbits, pentobarbital sodium a chlordiazepoxide proved effective in preventing seizures and death caused by 4-me-I administered in time. These two agents may be considered as antidotes of the neur logical signs induced by 4-me-I. The depressing effect of 4-me-I on spontaneous mot activity of mice and the heart rate of rabbits found in this work, and the reported i crease in peristaltic movements of intestines and the lowering of blood pressure 4-me-I (Supniewski, 1928; Yamamoto, 1941) indicate that the restlessness and defined "hysteria" are not of sympathomimetic nature. These symptoms described cattle intoxicated with ammoniated products are more closely related to convulsa drugs in sublethal doses.

By comparing the sensitivities of day-old chicks, mice, and rabbits to 4-me-I, it c be concluded that mice are best suited for testing the ammoniated agricultural p products which might contain 4-me-I. This is due to their low weight which requi less test material, their availability and their sensitivity to 4-me-I and other convulsant

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TOXICOLOGY AND APPLIED PHARMACOLOGY 17, 244–249 (1970)

Pharmacology of Alkyl and Hydroxyalkylpyrazines

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Pharmacology of Alkyl and Hydroxyalkylpyrazines. NISHIE, K., WAISS, A. C., JR., and KEYL, A. C. (1970). *Toxicol. Appl. Pharmacol.* 17, 244–249. The following by-products formed in the ammoniation of glucose were studied pharmacologically: pyrazine; 2-methylpyrazine; 2-hydroxymethylpyrazine; 2,3-dimethylpyrazine; 2,5-dimethylpyrazine; 2,6-dimethylpyrazine; 2-hydroxy-5-methylpyrazine; 2-methyl-5-arabotetrahydroxybutylpyrazine; 2-methyl-6-arabotetrahydroxybutylpyrazine; 4(5)-methylimidazole (4-me-I); 4-hydroxymethylimidazole; and 4-arabotetrahydroxybutylimidazole. Of these and other by-products, only 4-me-I had convulsant activity and the ability to induce in laboratory animals the signs observed in cattle fed toxic ammoniated molasses. Other derivatives of imidazole (hydroxymethyl and arabotetrahydroxybutyl) were pharmacologically inactive at a dose of 2 g kg in mice. With the exception of 4-me-I, all compounds in this series were relatively nontoxic. Mono- and dimethylpyrazines showed weak central nervous depressant activity (hypnotic and anticonvulsant). 2,3-Dimethylpyrazine was the most potent in this regard, but had only approximately 1/13 of the hypnotic activity of phenobarbital sodium and 1/21 of the anticonvulsant potency of phenobarbital against 4-me-I-induced seizures. Pyrazines with either OH, CH_2OH or $—(CHOH)_3—CH_2OH$ substituents were inactive as hypnotics and anticonvulsants and, in addition, were relatively nontoxic.

In a previous paper (Nishie *et al.*, 1969), the toxicology of the convulsant compound 4-methylimidazole was reported, and it was considered a possible etiological factor in the development of the neurological syndrome suffered by cattle fed toxic ammoniated molasses. The precise role of 4-methylimidazole, however, was obscured by the fact that in addition to 4-methylimidazole, a series of pharmacologically unclassified methyl, hydroxymethyl, dimethyl, arabotetrahydroxybutyl imidazoles and pyrazines were present in the ammoniated product. Some of these rare imidazole and pyrazine derivatives have been synthesized recently, and this has permitted the continuation of the current toxicologic study of by-products formed by the interaction of ammonia with reducing sugars.

METHODS

Male albino mice weighing 18–25 g were used to determine the anticonvulsant activity of the alkylpyrazines by measuring their ability to prevent: (a) the tonic extension component of maximal electroshock (MES) (Swinyard *et al.*, 1952), (b) convulsions induced by 100 mg kg, sc, pentylene tetrazole (Met) (Toman *et al.*, 1952), and (c) convulsions induced by 200 mg kg, ip, of 4-methylimidazole (4-me-I). The apparatus used pre-

...ce the ...ired electroshock current (50 mA) through corneal electrodes was a minor ...ation ...hat described by Woodbury and Davenport (1952). All circuit parameters ...mained ...changed with the exception of the timing circuit which was replaced by a ...ss Instrument Co.[1] S-4 stimulator in order to provide a 24-V pulse of 200 msec ...ration to the relay which activates the primary of the high voltage transformer. The ...ses of alkylpyrazines required to protect 50% of the mice against MES (MES ED50), ...ntylenetetrazole (Met ED50), and 4-me-I (4-me-I ED50) were determined by treating ...groups of 10 mice with graded doses of alkylpyrazines before MES, Met, or 4-me-I ...allenge and using, for purposes of computation of ED50 values, the log probit method ...Miller and Tainter (1944). The hypnotic effect of alkylpyrazines was determined by ...ng 3 groups of 10 mice subjected to graded doses of test compound in order to pro...e a loss of righting reflex (>1 min) in 50% of the animals (HD50). The effect of 2,3-...methylpyrazine on the electroencephalogram (EEG) was tested using rabbits im...anted with chronic EEG electrodes by the method previously described (Nishie *et al.*, ...). The LD50 values of the pyrazine and imidazole derivatives were determined ...ng 3 groups of 10 mice given graded doses of test compound, and observation for a ...eek period. All alkylpyrazines were dissolved in saline prior to administration and ...e route of administration in all cases was ip.

RESULTS

The majority of the compounds formed during the ammoniation of glucose are listed ...Table I, and their pharmacologic data are summarized in Table 2. Phenobarbital was ...luded as a reference compound for purpose of comparison. It is evident that these ...mpounds, with the exception of 4-me-I are relatively nontoxic. 4-Me-I was the only ...mpound possessing convulsant activity, whereas other derivatives of imidazole with ...ther hydroxymethyl or arabotetrahydroxybutyl substituents were neither convulsant ...r lethal at a 2 g/kg dose, and did not produce any untoward visible effects in mice ...ring a 1-week period of observation.

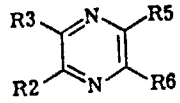
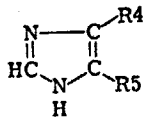
Hypnotic Properties of the Alkylpyrazines

Altho... pyrazine itself was not hypnotic (2 g/kg), its mono and dimethyl derivatives ...re wea... hypnotic; 2,3-dimethylpyrazine was the most potent compound in this ...ries, ...gh it had only about 1/13 of the activity of the phenobarbital sodium. The ...tio of ... 50/HD50 of the alkylpyrazines varied from 2.5 to 1.3; 2,3-dimethylpyrazine ...d the ... safety margin with a ratio of 2.5, and 2,6-dimethylpyrazine had the lowest ...afety ... in with a ratio of 1.3. The phenobarbital ratio was 2.9. A limited number ...f experiments using rabbits showed that iv administered 2,3-dimethylpyrazine ...roduced EEG changes similar to those observed with barbiturates, the chief dif...ference consisting of a short duration of sleep at relatively high doses (>200 mg/kg) of ...dimeth...lpyrazine probably due to rapid elimination of the compound through the ...ngs (indicated by the strong characteristic odor in the expired air). The derivatives of ...razine and imidazole with hydroxy, hydroxymethyl or arabotetrahydroxybutyl ...bstituent groups were devoid of hypnotic and anticonvulsant actions (Table 2).

[1] Reference to a company or product name does not imply approval or recommendation of the ...oduct by the U.S. Department of Agriculture to the exclusion of others that may be suitable.

TABLE 1

COMPOUNDS FORMED DURING AMMONIATION OF GLUCOSE AND TESTED FOR NEUROPHARMACOLOGIC ACTIVITY

No.	Compound	MW	R2	R3	R5	R6
1	Pyrazine 	80	H	H	H	H
2	2-Methylpyrazine	94	CH_3	H	H	H
3	2-Hydroxymethylpyrazine	110	CH_2OH	H	H	H
4	2,3-Dimethylpyrazine	108	CH_3	CH_3	H	H
5	2,5-Dimethylpyrazine	108	CH_3	H	CH_3	H
6	2,6-Dimethylpyrazine	108	CH_3	H	H	CH_3
7	2-Hydroxy-5-methylpyrazine	110	OH	H	CH_3	H
8	2-Methyl-5-arabotetrahydroxybutylpyrazine	214	CH_3	H	$-(CHOH)_3-CH_2OH$	H
9	2-Methyl-6-arabotetrahydroxybutylpyrazine	214	CH_3	H	H	$-(CHOH)_3-CH_2OH$
10	4-Methylimidazole (4-me-I)[a] 	82	$R4 = CH_3$			
11	4-Hydroxymethylimidazole[a]	118	$R4 = CH_2OH$			
12	4-Arabotetrahydroxybutylimidazole[a]	188	$R4 = -(CHOH)_3-CH_2OH$			
13	Phenobarbital sodium	254				

[a] Substitutions are either in position 4 or 5 of the imidazole molecule.

TABLE 2

PHARMACOLOGIC ACTIVITIES IN MICE OF COMPOUNDS LISTED IN TABLE 1[a]

Compound No.	LD50 ± SE (mg/kg)	CD50 ± SE (mg/kg)	HD50 ± SE (mg/kg)	MES ED50 ± SE (mg/kg)	Met ED50 ± SE (mg/kg)	4-me-I ED50 ± SE (mg/kg)
1	>2000	—	>2000	1000 ± 51	1360 ± 78	322 ± 26
2	1820 ± 60	—	1250 ± 25	580 ± 23	530 ± 64	130 ± 18
3	>2000	—	>2000	>2000	>2000	1320 ± 200
4	1390 ± 20	—	540 ± 26	355 ± 24	330 ± 17	130 ± 11
5	1350 ± 23	—	880 ± 23	575 ± 43	540 ± 19	257 ± 24
6	1080 ± 14	—	840 ± 41	480 ± 45	385 ± 20	114 ± 15
7	1300 ± 64	—	>2000	>2000	>2000	>2000
8	>1560[b]	—	>1560	—	—	—
9	>316[b]	—	>316	—	—	—
10	165 ± 3	155 ± 5	—	—	—	—
11	>2000	—	>2000	>2000	>2000	>2000
12	>2000	—	>2000	>2000	>2000	>2000
13	270 ± 5.9	—	94 ± 5	15.2 ± 2.2	27.5 ± 1.5	14 ± 3.4

[a] CD50, dose to produce convulsion in 50% of mice; HD50, hypnotic dose, dose to produce loss of righting reflex in 50% of mice (>1 min); MES ED50, dose to protect 50% of mice against maximal electroshock (MES); Met ED50, dose to protect 50% of mice against convulsions induced by 100 mg/kg sc pentylenetetrazol (Metrazol); 4-me-I ED50, dose to protect 50% of mice against convulsions induced by 200 mg/kg ip 4-methylimidazole.

[b] Highest dose tested without producing death or loss of righting reflex.

Anticonvulsant Properties of the Alkylpyrazines

Convulsions induced in mice by MES, pentylenetetrazole (Met) and 4-me-I were prevented by pyrazine and its hypnotic derivatives. Pyrazine was the only compound in this series without hypnotic activity, but possessed anticonvulsant activity against MES, Met, and 4-me-I. 2-Hydroxymethylpyrazine was inactive as a hypnotic, but acted as a weak anticonvulsant agent against seizure induced by 4-me-I, but not against MES and pentylenetetrazole-induced seizures. The anticonvulsant effects of the pyrazines were achieved at dose levels equivalent to 29–66% of their corresponding HD50 values. Comparison with the anticonvulsant potency of phenobarbital sodium reveals that pyrazine and the methylpyrazines are very weak. The most active anticonvulsant compound in this series, 2,3-dimethylpyrazine, had about 1/54 the potency of phenobarbital against MES seizures, 1/27 the potency against pentylenetetrazole, and 1/21 the potency against 4-me-I-induced seizures.

DISCUSSION

The alkylpyrazines described in this paper are produced by a number of different methods: (a) ammoniation of glucose or reducing sugar-containing agricultural by-products (Hough *et al.*, 1952; Wiggins, 1956; Wiggins and Wise, 1956; Jezo, 1966), (b) heating a mixture of amino acids such as aspartic acid, glutamic acid, or asparagine with carbohydrates (Newell *et al.*, 1967; Koehler *et al.*, 1969; Dawes and Edwards, 1966); or (c) heating foods containing amino acids and sugars such as peanuts, coffee, cocoa, and potatoes. These alkylpyrazines constitute part of the aroma profile of these foods.

Pyrazine does not have a distinctive odor; however, its derivatives have characteristic odors, e.g., 2,3-dimethylpyrazine has the aroma of green bananas, while 2-hydroxy-5-methylpyrazine in aqueous solution resembles that of ripe bananas. 2,3-Dimethylpyrazine was identified as part of the aroma profile of coffee and chocolate (Viani *et al.*, 1965; Gianturco *et al.*, 1956; Rizzi, 1967); 2,5-dimethylpyrazine of coffee, chocolate, roasted peanuts, and potato chips (Gianturco *et al.*, 1956; Rizzi, 1967; Mason *et al.*, 1960; Deck and Chang, 1965); 2,6-dimethylpyrazine was found in coffee and chocolate (Gianturco *et al.*, 1956; Rizzi, 1967), and 2-methylpyrazine was identified in chocolate (Rizzi, 1967).

These pyrazines of relatively low molecular weight have not previously been investigated pharmacologically, with the exception of pyrazine itself, which was tested as an anthelmintic compound (Cavier, 1966). The pharmacologic properties shown in Table 2 indicate that the alkylpyrazines are central depressants with weak hypnotic and anticonvulsant characteristics and possess a low order of toxicity.

This investigation has provided strong evidence that the chief toxic factor in ammoniated molasses is 4-methylimidazole, because it appears to be the only compound formed during ammoniation capable of reproducing the signs, observed in cattle fed toxic ammoniated products, in small laboratory animals (Nishie *et al.*, 1969). Wiggins (1956) reported that during ammoniation, conditions of high temperature and high pressure favored pyrazine formation, but indicated nothing about 4-methylimidazole formation. If pyrazine formation is favored with concurrent reduction in the formation of 4-methylimidazole, ammoniation processes should be carried out under conditions of high pressure and temperature in order to produce a nontoxic ammoniated feed. This feed

should contain sufficient quantities of nontoxic, anticonvulsant alkylpyrazines to prevent convulsions or preconvulsive excitation due to 4-methylimidazole formed simultaneously during the ammoniation. It is indeed curious that pharmacologically antagonistic methylpyrazines and methylimidazoles occur simultaneously in the ammoniation of glucose and that the toxicity of the mixture apparently depends on the relative ratios of these materials.

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COMPOSITION OF CARAMEL PREPARED BY HEATING SUGARS

During the manufacture of cane sugar, caramel which is a mixture of nitrogen-free organic compounds,[1] is produced due to overheating of sucrose or its decomposition products.[2,3] It not only imparts colour to the sugar crystals, but also inhibits the rate of crystallisation.[4] Caramel is used in food industry as a colouring and flavouring agent of alcoholic drinks and sweetmeats.[5,6] Very little work has been done on the composition of the caramel. Schumaker and Buchanan[7] reported that caramel contained a few anhydrides with empirical formulæ, $C_{12}H_{20}O_{10}$, $C_{24}H_{36}O_{18}$ and $C_{36}H_{50}O_{25}$. It is believed[8] that the composition of caramel varies not only with the pH and temperature of heating but also with the type of sugar used for its preparation. The present communication reports chromatographic studies on the composition of caramel prepared from different sugars.

Pure glucose, fructose, sucrose, maltose (B.D.H. samples) and liquid glucose (from Ravalgaon Sugar Farm, Ltd.), were used as raw materials; from these, caramel was prepared by two methods: (*a*) heating the sugar as such at 200° C., and (*b*) heating the sugar in solution phase in presence of alkali at 100° C. The details of the procedure were given elsewhere.[9] Caramel was separated from the system by precipitation with alcohol. The caramels obtained from different sugars were examined chromatographically using Whatman filter-paper No. 1 and *n*-butanol-ethanol-water mixture as the solvent.[10]

Fig. 1 gives a typical set of chromatograms indicating the constituents of the caramels; in this, series A refer to the caramels prepared by method *b*. It was interesting to note that there existed four reducing compounds with roughly the same R_f (× 100) values of 6·5, 4·35, 1·4, 0 (reference value—levulose 13·6) in all the caramels prepared from different sugars. Contrary to the general belief was the observation that whether a single sugar or a mixture of sugars was employed for caramelisation, the same four reducing compounds were detected. This is due presumably to the production before caramelisation of an intermediate equilibrium mixture of glucose, fructose and mannose where sugars were heated in alkaline solutions as envisaged in the Lobry de Bruyn and Alberde van Ekenste in transformation.[11] The observation of a spot with R_f value of 0 (on the reference line) indicated the presence of one (or more) reducing substance(s)* of relatively high molecular weight.

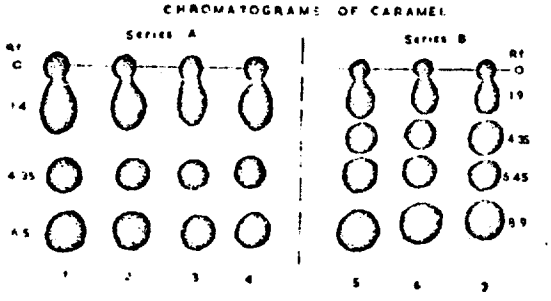

FIG. 1. Chromatographic Studies of the Composition of Caramel. Series A refers to the caramels prepared by heating sugars with alkali in the solution phase and series B to those prepared by heating sugars as such. 1 and 5 from dextrose; 2 and 6 from levulose; 3 from maltose; 4, liquid glucose; and 7, mixture of sugars. Time of running the chromatograms for series A was 96 hr. and for series B, 72 hr. Temperature 35° C. The standard reference value employed for computation of R_f values of the spots of caramel was taken as 13·6 for levulose (not shown in Fig. 1).

Chromatograms in Fig. 1 B which refers to the analysis of caramel prepared by heating sugars as such (method *a*) indicated the presence of another reducing compound with R value of 8·9, in addition to the four detected in caramels prepared by the method *b* (Fig. 1 A). As in method *b*, the same number of reducing compounds with equal R_f values were noticed whatever may be the sugar employed. This observation points out that the van Ekenstein transformation known to take place in heated alkaline sugar solutions, appears to take place when sugars are heated as such.

The results in Fig. 1 show clearly that the composition of the caramel varies not with the sugar used, but with the method employed for its preparation.

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* This could not be separated from the reference line even after running the chromatograms for 5–6 days.

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